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## OB PROTEIN RECEPTOR AND RELATED COMPOSITIONS AND METHODS

### FIELD OF THE INVENTION

5 The present invention relates to OB protein receptors, related compositions and methods of making and using such receptors and related compositions.

### BACKGROUND

10 Although the molecular basis for obesity is largely unknown, the identification of the "OB gene" and protein encoded ("OB protein") has shed some light on mechanisms the body uses to regulate body fat deposition. Zhang et al., *Nature* 372: 425-432 (1994); see  
15 also, the Correction at *Nature* 374: 479 (1995). The OB protein is active in vivo in both *ob/ob* mutant mice (mice obese due to a defect in the production of the OB gene product) as well as in normal, wild type mice. The biological activity manifests itself in, among other  
20 things, weight loss. See generally, Barinaga, "Obese" Protein Slims Mice, *Science* 269: 475-476 (1995). See PCT International Publication Number WO 96/05309, "Modulators of Body Weight, Corresponding Nucleic Acids and Proteins, and Diagnostic and Therapeutic Uses  
25 - Thereof," herein incorporated by reference.

The other biological effects of OB protein are not well characterized. It is known, for instance, that in *ob/ob* mutant mice, administration of OB protein results in a decrease in serum insulin levels, and serum  
30 glucose levels. It is also known that administration of OB protein results in a decrease in body fat. This was observed in both *ob/ob* mutant mice, as well as non-obese normal mice. Pelleymounter et al., *Science* 269: 540-543 (1995); Halaas et al., *Science* 269: 543-546 (1995). See

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also, Campfield et al., Science 269: 546-549 (1995) (Peripheral and central administration of microgram doses of OB protein reduced food intake and body weight of *ob/ob* and diet-induced obese mice but not in *db/db* obese mice.) In none of these reports have toxicities been observed, even at the highest doses.

Despite the promise of clinical application of the OB protein, the mode of action of the OB protein *in vivo* is not clearly elucidated, in part due to the absence of information on the OB receptor. High affinity binding of the OB protein has been detected in the rat hypothalamus, reportedly indicating OB receptor location. Stephens et al., Nature 377: 530-532 (1995). The *db/db* mouse displays the identical phenotype as the *ob/ob* mouse, i.e., extreme obesity and Type II diabetes; this phenotype is thought to be due to a defective OB receptor, particularly since *db/db* mice fail to respond to OB protein administration. See Stephens et al., supra.

Identification of the OB protein receptor is key in determining the pathway of signal transduction. Moreover, identification of the OB protein receptor would provide powerful application in diagnostic uses, for example, to determine if individuals would benefit from OB protein therapy. Furthermore, the OB receptor could be a key component in an assay for determining additional molecules which bind to the receptor and result in desired biological activity. Further, such soluble receptor could enhance or alter the effectiveness of OB protein (or analog or derivative thereof).

#### SUMMARY OF THE INVENTION

The present invention relates to a novel class of protein receptors, herein denominated "OB protein receptors" or "OB receptors", which are thought to selectively bind OB protein. As such, the novel OB

receptor family is provided, as well as novel members of such family. Also provided are nucleic acids, vectors and host cells containing such nucleic acids, related antisense nucleic acids, molecules which selectively bind to the OB protein receptor, and related compositions of matter, such as OB receptor protein/OB protein complexes. In other aspects, the present invention relates to methods of using the above compositions, such as therapeutic and/or diagnostic methods, and methods for preparing OB receptor ligands.

DETAILED DESCRIPTION

A novel family of OB receptors is provided. This novel family resulted from identification of a PCR fragment isolated from a human liver cell cDNA library. The original PCR fragment, from which primers were isolated, contained a "WSXWS" motif, common to cytokine receptors. As illustrated by the working examples below, using this fragment four members of this OB protein receptor family have been identified. These members, herein designated as "A", "B", and "C", and "D" are identical at amino acid position 1-891 (using the numbering of Seq. ID No. 1), but diverge at position 892 through the C-terminus. They vary in length at the C-terminus beyond amino acid 891, and the different forms appear to have different tissue distribution.

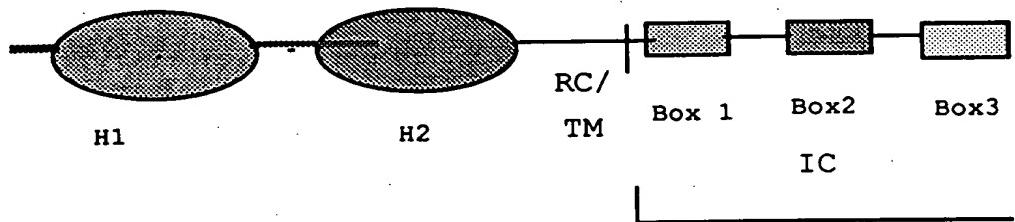
Using hydrophobicity analysis, the leader sequence is likely to comprise amino acids (Seq. ID. No. 1) 1-21, 1-22, or 1-28. The first amino acid of the mature protein is likely to be 22 (F), 23 (N) or 29 (T). Most likely, based on analysis of eucaryotic cell expression (CHO cell expression see Example 8, infra), the first amino acid of the mature protein is 22(F). The beginning of the transmembrane domain appears to be located at position 840 (A) or 842 (L). The end of the transmembrane domain appears to be located at position

862 (I), 863 (S) or 864 (H). Thus, based on predictions from hydrophobicity analysis, for OB protein binding, at a minimum what is needed is the extracellular domain of the mature protein, amino acids 22, 23 or 29 through amino acids 839 (D) or 841 (G). Therefore, the present class of OB receptor proteins includes those having amino acids (according to Seq. ID No. 1):

- (a) 1-896;
- (b) 22-896;
- 10 (c) 23-896;
- (d) 29-896;
- (e) 1-839;
- (f) 22-839;
- (g) 1-841;
- 15 (h) 22-841;
- (i) 23-841;
- (j) 29-841;
- (l) 1-891;
- (m) 22-891;
- 20 (n) 23-891;
- (o) 29-891;
- (p) the amino acids of subparts (l) through (o) having the C-terminal amino acids selected from among:
  - 25- (i) OB receptor B (Seq. ID No. 3) positions 892-904;
  - (ii) OB receptor C (Seq. ID No. 5) positions 892- 958; and,
  - (iii) OB receptor D (Seq. ID No. 7)
- 30 positions 892-1165;
  - (q) amino acids of subparts b, c, d, f, g, i, j, k, m, n, o, and any of (p) lacking a leader sequence, which have an N-terminal methionyl residue.
- 35 Also provided herein is what is thought to be a human splice variant of a soluble OB receptor. This

splice variant includes the extracellular domain at least up to amino acid 798 (of Seq. ID No. 1, for example) and has a unique 6 amino acid C-terminus at positions 799-804: G K F T I L.

5       The functional domains of the OB receptor may be predicted using the information contained in Bazan et al., PNAS-USA 87: 6934-6938 (1990) (incorporated herein by reference). For the present OB receptor, there are  
10      two hematopoietin domains, a random coil region, the transmembrane domain, and the intracellular domain. The overall geography may be illustrated as follows:



15      Using the information provided by Bazan,  
supra, the domains may be predicted, with essentially an error of approximately plus or minus three base pairs (as applied to all amino acid location specified for purposes of identifying the Bazan predicted domains).  
20      The precise locations may be determined empirically by methods known in the art, such as preparing and expressing modified recombinant DNAs. The structural characteristics are thought to be important for maintaining the structural integrity of the molecule, and therefore, to the extent that such structure is  
25      important for function, for functional characteristics as well.

30      The hematopoietin domains (H1 and H2) are thought to have two fibronectin type 3 repeats each, one set of paired cysteine residues each (thought to form a disulfide bridge), and one "WSXWS box" (referring to the

single letter amino acid abbreviation, with "X" being any amino acid). The fibrinectin type 3 domains may be identified by location of a double proline ("PP"), which marks the beginning of the second fibronectin type 3 repeat; the actual beginning of such second fibronectin type 3 repeat is likely to begin about 3 amino acids upstream of that double proline.

The first hematopoietin domain is likely to begin at amino acid 123 (using the numbering according to Seq. ID No. 1, for example), which is an isoleucine residue (I). The last amino acid of the hematopoietin domain is likely to be amino acid 339, which is a lysine (K) residue. The two fibronectin type 3 repeats are likely to be located at (about) amino acids 123 through 235 and 236 through 339. There is a single pair of cysteine residues which likely form a disulfide bridge, located at position 131 and position 142. The "WSXWS box" is located at position 319 through 323.

The second hematopoietin domain is likely to begin at position 428, which is an isoleucine (I) and end at position 642 which is a glycine (G). The paired fibronectin type 3 repeats are located at about position 428 through position 535 and about position 536 through about position 642. One pair of cysteines is located at position 436 and position 447, and the second pair is located at position 473 and 488. The "WSXWS box" is located at position 622-626.

Between the first and the second hematopoietin domain (amino acids 339-428, approximately) is a region of unknown functional significance.

The random coil domain ("RC" between the H2 and the transmembrane domain, "TM") is likely to begin at the amino acid following the end of the second hematopoietin domain, and is likely to end at the beginning of the transmembrane domain. This is likely to be from about amino acid 642 through amino acid 839

or 841 (with the transmembrane domain beginning at position 840 (A) or 842 (L)). The intracellular domain ("IC") is likely to begin at position 861 (L), 862 (I), 863 (S) or 864 (H).

5           The intracellular domain ("IC") contains three regions, or "boxes," thought to participate in signal transduction (two "JAK" boxes and a single "STAT" box, "Box 1", "Box 2", and "Box 3"). With respect to the numbering of the amino acid positions of the "D" form of  
10 the OB receptor (Seq. ID No.7, below), box 1 is located at amino acid 871 (F) through 878 (P). Box 2 is located at approximately amino acid number 921 (I) through 931 (K). Box 3 on the "D" form is located at approximately position 1141 through 1144 (amino acids YMPQ, as  
15 the "STAT" box is typically a conserved region of "YXXQ" wherein "X" designates any amino acid). The intracellular domain is thought to be responsible for signal transduction. One possible mode of action is via phosphorylation of various residues. See Ihle et al.,  
20 Cell 84: 331-334 (1996) (Review article, herein incorporated by reference.)

One possible mode of action is that upon ligand binding (here, OB protein binding), the OB receptor dimerizes with another receptor. A kinase ("JAK") binds to box 1, and becomes phosphorylated.  
25- (The JAK may already be bound prior to dimerization.) Also, "STATs" bind to box 3 and become phosphorylated on a specific tyrosine. It is thought that this phosphorylation results, probably indirectly, in DNA binding protein production, which results in altered DNA transcription, and therefore altered expression. As seen below in Example 6, one measurement of the capability of an OB receptor to transduce signal is the degree of phosphorylation of JAK/STAT molecules.

35           The C-terminus region is intracellular (of cell-bound OB receptor). The differences in the C-

terminus among members of the present OB receptor family may result in differences in signal transduction among the species. Thus, the present OB receptors include at least the extracellular domain which is important for OB protein ligand binding. Nucleic acids encoding the present OB receptors, vectors, and host cells are also provided for herein.

The extracellular domain may be modified and still retain the function of ligand binding,

particularly by one or more of the following modifications: (a) the random coil domain (as indicated above, occurring downstream of the second hematopoietic domain through the beginning of the transmembrane domain) may be deleted (this may be approximately positions 642 through 839 or 841); (b) the "WSXWS" box may be modified by (i) substitution of the first serine with another amino acid, particularly conserved in terms of hydrophobicity and/or charge, such as a glycine; (ii) the last serine may be substituted with another amino acid, such as a threonine; (iii) the first tryptophan may be substituted with another amino acid, for example, a tyrosine.

Human genomic DNA encoding OB receptor protein is also provided herein. The genomic DNA has been

localized to human chromosome 1P31, which is believed to correspond to mouse chromosome 4, the location of the mouse *db* locus.

Tissue distribution analysis demonstrates the presence of OB receptor nucleic acids is fairly

ubiquitous, and particularly noted in the liver. It is also observed in the ovary, and heart; and, to a lesser extent, in small intestine, lung, skeletal muscle, kidney, and, to an even lesser extent, spleen, thymus, prostate, testes, placenta and pancreas (Example 2, below). There may also be one or more forms of the OB receptor present in serum, such as soluble OB receptor,

which may be complexed to one or more forms of the OB protein.

Amino Acid Sequences and Compositions

According to the present invention, novel OB protein receptors and DNA sequences coding for all or part of such OB receptors are provided. The present invention provides purified and isolated polypeptide products having part or all of the primary structural conformation (i.e., continuous sequence of amino acid residues) and one or more of the biological properties (e.g., immunological properties and *in vitro* biological activity) and physical properties (e.g., molecular weight) of naturally-occurring mammalian OB receptor including allelic variants thereof. The term "purified and isolated" herein means substantially free of unwanted substances so that the present polypeptides are useful for an intended purpose. For example, one may have a recombinant human OB receptor substantially free of human proteins or pathological agents. These polypeptides are also characterized by being a product of mammalian cells, or the product of chemical synthetic procedures or of procaryotic or eucaryotic host expression (e.g., by bacterial, yeast, higher plant, insect and mammalian cells in culture) of exogenous DNA sequences obtained by genomic or cDNA cloning or by gene synthesis. The products of expression in typical yeast (e.g., *Saccharomyces cerevisiae*), insect, or procaryote (e.g., *E. coli*) host cells are free of association with any mammalian proteins. The products of expression in vertebrate (e.g., non-human mammalian (e.g. COS or CHO) and avian) cells are free of association with any human proteins. Depending upon the host employed, and other factors, polypeptides of the invention may be glycosylated with mammalian or other eucaryotic carbohydrates or may be non-glycosylated. One may modify

the nucleic acid so that glycosylation sites are included in the resultant polypeptide. One may choose to partially or fully deglycosylate a glycosylated polypeptide. Polypeptides of the invention may also include an initial methionine amino acid residue (at position -1 with respect to the first amino acid residue of the mature polypeptide).

In addition to naturally-occurring allelic forms of OB receptor, the present invention also embraces other OB receptor products such as polypeptide analogs of OB receptor and fragments of OB receptor. Following the procedures of the above noted published application by Alton et al. (WO 83/04053), one can readily design and manufacture genes coding for microbial expression of polypeptides having primary conformations which differ from that herein specified for in terms of the identity or location of one or more residues (e.g., substitutions, terminal and intermediate additions and deletions). Alternately, modifications of cDNA and genomic genes may be readily accomplished by well-known site-directed mutagenesis techniques and employed to generate analogs and derivatives of OB receptor. Such products would share at least one of the biological properties of mammalian OB receptor but may differ in others. As examples, projected products of the invention include those which are foreshortened by e.g., deletions; or those which are more stable to hydrolysis (and, therefore, may have more pronounced or longer lasting effects than naturally-occurring); or which have been altered to delete one or more potential sites for glycosylation (which may result in higher activities for yeast-produced products); or which have one or more cysteine residues deleted or replaced by, e.g., alanine or serine residues and are potentially more easily isolated in active form from microbial systems; or which have one or more tyrosine residues

replaced by phenylalanine; or have an altered lysine composition (such as those prepared for purposes of derivatization). Included are those polypeptides with amino acid substitutions which are "conservative" 5 according to acidity, charge, hydrophobicity, polarity, size or any other characteristic known to those skilled in the art. See generally, Creighton, Proteins, W.H. Freeman and Company, N.Y., (1984) 498 pp. plus index, passim. One may make changes in selected amino acids so 10 long as such changes preserve the overall folding or activity of the protein, (see Table 1, below). Small amino terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or a small extension that facilitates 15 purification, such as a poly-histidine tract, an antigenic epitope or a binding domain, may also be present. See, in general Ford et al., Protein Expression and Purification 2: 95-107, 1991, which is herein incorporated by reference.

Table 1  
Conservative Amino Acid Substitutions

|              |   |
|--------------|---|
| Basic:       | arginine<br>lysine<br>histidine                         |
| Acidic:      | glutamic acid<br>aspartic acid                          |
| Polar:       | glutamine<br>asparagine                                 |
| Hydrophobic: | leucine<br>isoleucine<br>valine                         |
| Aromatic:    | phenylalanine<br>tryptophan<br>tyrosine                 |
| Small:       | glycine<br>alanine<br>serine<br>threonine<br>methionine |

5           Also comprehended are polypeptide fragments  
          duplicating only a part of the continuous amino acid  
          sequence or secondary conformations within OB receptor,  
          which fragments may possess one activity of mammalian  
          (particularly human) OB receptor (e.g., immunological  
10          activity) and not others (e.g., OB protein binding  
          activity).

15          Of applicability to OB receptor fragments and  
          polypeptide analogs of the invention are reports of the  
          immunological activity of synthetic peptides which  
          substantially duplicate the amino acid sequence extant  
          in naturally-occurring proteins, glycoproteins and  
          nucleoproteins. More specifically, relatively low

molecular weight polypeptides have been shown to participate in immune reactions which are similar in duration and extent to the immune reactions of physiologically significant proteins such as viral antigens, polypeptide hormones, and the like. Included among the immune reactions of such polypeptides is the provocation of the formation of specific antibodies in immunologically active animals. See, e.g., Lerner et al., Cell 23: 309-310 (1891); Ross et al., Nature 294: 5

10 654-656 (1891); Walter et al., PNAS-USA 77: 5197-5200 (1890); Lerner et al., PNAS-USA, 78: 3403-3407 (1891); Walter et al., PNAS-USA 78: 4882-4886 (1891); Wong et al., PNAS-USA 79: 5322-5326 (1982); Baron et al., Cell 28: 395-404 (1982); Dressman et al., Nature 295: 185-160

15 (1982); and Lerner, Scientific American 248: 66-74 (1983). See, also, Kaiser et al. Science 223: 249-255 (1984) relating to biological and immunological activities of synthetic peptides which approximately share secondary structures of peptide hormones but may

20 not share their primary structural conformation. The present invention also includes that class of polypeptides coded for by portions of the DNA complementary to the protein-coding strand of the human cDNA or genomic DNA sequences of OB receptor i.e., "complementary

25 inverted proteins" as described by Tramontano et al. Nucleic Acid Res. 12: 5049-5059 (1984). Polypeptides or analogs thereof may also contain one or more amino acid analogs, such as peptidomimetics.

Thus, the present class of OB receptor proteins includes those having amino acids (according to Seq. ID No. 1):

- (a) 1-896;
- (b) 22-896;
- (c) 23-896;
- 35 (d) 29-896
- (e) 1-839;

- (f) 22-839;
- (g) 29-839;
- (h) 1-841;
- (i) 22-841;
- (j) 23-841;
- (k) 29-841;
- (l) 1-891;
- (m) 22-891;
- (n) 23-891;
- (o) 29-891;

10

- (p) the amino acids of subparts (l)

through (o) having the C-terminal amino acid sequence beginning at position 892 of OB receptor B (Seq. ID No. 3) or C (Seq. ID. No. 5);

15

(q) amino acids of subparts b, c, d, f, g, i, j, k, m, n, o, and any of (p) lacking a leader sequence, which have an N-terminal methionyl residue.

20

Also provided is a longer form of an OB receptor protein, herein denominated the "D" form, which has an amino acid sequence selected from among (according to Seq. ID No. 7):

25-

- (a) amino acids 1-1165;
- (b) amino acids 22-1165;
- (c) amino acids 23-1165;
- (d) amino acids 29-1165;
- (e) amino acids of subparts (b), (c) or (d) having an N-terminal methionyl residue.

As set forth above, one may prepare soluble receptor by elimination of the transmembrane and intracellular regions. Examples of soluble receptors include those set forth in Seq. ID Nos. 10 and 13. What is thought to be a native, secreted form of a soluble human OB receptor is also provided herein. This form of OB receptor protein has an amino acid sequence selected from among (according to Seq. ID No. 13):

- (a) amino acids 1-804;

- (b) amino acids 22-804;  
(c) amino acids 23-804;  
(d) amino acids 29-804; and,  
(e) amino acids of subparts (b), (c) or  
5 (d) having an N-terminal methionyl residue.

In addition, since the C-terminus region of the above polypeptides diverges at position 892 (with respect to Seq. ID Nos. 1, 3, 5, 7 and 13) one may desire to prepare only the polypeptides which are

- 10 divergent:  
(a) those having only amino acids 892-896  
of Seq. ID No. 1;  
(b) those having only amino acids 892-904  
of Seq. ID No. 3;  
15 (c) those having only amino acids 892-958  
of Seq. ID No. 5;  
(d) those having only amino acids 892-  
1165 of Seq. ID No. 7; and,  
(e) those having only amino acids 799-804  
20 of Seq. ID No. 13.

The above polypeptides which have an extracellular domain may be modified, as indicated above, and still retain the function of ligand binding. Such modification may include one or more of the  
25 following:

- (a) the random coil domain (as indicated above, occurring downstream of the second hematopoietic domain through the beginning of the transmembrane domain) may be deleted (this may be approximately  
30 positions 642 through 839 or 841);  
(b) the "WSXWS" box may be modified by  
(i) substitution of the first serine with another amino acid, particularly conserved in terms of hydrophobicity and/or charge, such as a glycine; (ii) the last serine  
35 may be substituted with another amino acid, such as a threonine; (iii) the first tryptophan may be

substituted with another amino acid, for example, a tyrosine.

Thus, the present polypeptides include (according to the numbering of Seq. ID No. 7):

- 5 (a) 1-896;
- (b) 22-896;
- (c) 23-896;
- (d) 29-896
- (e) 1-839;
- 10 (f) 22-839;
- (g) 29-839;
- (h) 1-841;
- (i) 22-841;
- (j) 23-841;
- 15 (k) 29-841;
- (l) 1-891;
- (m) 22-891;
- (n) 23-891;
- (o) 29-891;
- 20 (p) the amino acids of subparts (l) through (o) having the C-terminal amino acids selected from the C-terminal amino acids of OB receptor B (Seq. ID No. 3), C (Seq. ID. No. 5) and D (Seq ID No. 7);  
(q) the amino acids (according to Seq. ID 25- No. 13) selected from the group consisting of 22-804; 23-804 and 29-804;  
(r) amino acids of subparts b, c, d, f, g, i, j, k, m, n, o, any of (p) lacking a leader sequence, and (q) which have an N-terminal methionyl residue; and  
30 (s) amino acids of subparts (a) through (r) which above having at least one of the following modifications:  
(i) for amino acids of subparts (a) through (p) and those of subpart (r) which are not amino acids according to subpart (q), deletion of (or

substitution of amino acid(s) or other modifications of a random coil domain sequence selected from

(a) 640 through 839 (using the numbering according to Seq. ID No. 1);

5

(b) 641 through 839;

(c) 642 through 839;

(d) 640 through 841;

(e) 641 through 841; and

(f) 642 through 841;

10

(ii) for amino acids of subpart (q)

and those of subpart (r) which contain the sequence of subpart (q), deletion of of (or substitution of amino acid(s) or other modifications of) a random coil domain sequence selected from among:

15

(a) 640 through 804;

(b) 641 through 804; and,

(c) 642 through 804;

and,

(iii) modification of a "WSXWS"

20 sequence which is

(a) substitution of the first serine with another amino acid, particularly conserved in terms of hydrophobicity and/or charge, such as a glycine;

25-

(b) substition of the last

serine with another amino acid, such as a threonine;

and

(c) substitution of the first tryptophan with another amino acid, for example, a

30 tyrosine.

One may modify the OB receptor to create a fusion molecule with other peptide sequence. For example, if one desired to "tag" the OB receptor with an immunogenic peptide, one could construct a DNA which would result in such fusion protein. The tag may be at the N-terminus. Also, since it is apparent that the

35

C-terminus is not necessary for ligand binding activity, one may chemically modify the C-terminus of, for example, a soluble OB receptor. One may desire, for example, a preparation whereby one or more polymer molecules such as polyethylene glycol molecules are attached. Thus, another aspect of the present invention is chemically modified OB receptor protein (also further described infra).

An example of such "tag" is provided herein using the C-terminus of a recombinant soluble OB receptor. Seq. ID No. 12 provides a "FLAG-tag" version of such soluble OB receptor (the nucleic acid sequence is provided, which may be transcribed to prepare the polypeptide). Such "FLAG-tag" may also be attached to the N-terminus or other region of an OB receptor protein. This type of "tagging" is useful to bind the protein using reagents, such as antibodies, which are selective for such tag. Such binding may be for detection of the location or amount of protein, or for protein capturing processes where, for example, an affinity column is used to bind the tag, and thus the desired protein. Other types of detectable labels, such as radioisotopes, light-emitting (e.g., fluorescent or phosphorescent compounds), enzymatically cleavable, detectable antibody (or modification thereof), or other substances may be used for such labelling of the present proteins. Detecting protein via use of the labels may be useful for identifying the presence or amount of OB receptor protein or a compound containing such protein (e.g., OB protein complexed to OB receptor). Moreover, such labelled protein may be useful for distinguishing exogenous OB receptor protein from the endogenous form.

Nucleic Acids

Novel nucleic acid sequences of the invention include sequences useful in securing expression in prokaryotic or eucaryotic host cells of polypeptide products having at least a part of the primary structural conformation and one or more of the biological properties of recombinant human OB receptor. The nucleic acids may be purified and isolated, so that the desired coding region is useful to produce the present polypeptides, for example, or for diagnostic purposes, as described more fully below. DNA sequences of the invention specifically comprise: (a) any of the DNA sequences set forth in Seq. ID No. 2, 4, 6, 8, 9, 11, 12, and 14 (and complementary strands); (b) a DNA sequence which hybridizes (under hybridization conditions disclosed in the cDNA library screening section below, using the 300 bp PCR fragment as described to selectively hybridize to a cDNA encoding an OB receptor protein in a human liver cDNA library, or equivalent conditions or more stringent conditions) to the DNA sequence in subpart (a) or to fragments thereof; and (c) a DNA sequence which, but for the degeneracy of the genetic code, would hybridize to the DNA sequence in subpart (a). Specifically comprehended in parts (b) and (c) are genomic DNA sequences encoding allelic variant forms of human OB receptor and/or encoding OB receptor from other mammalian species, and manufactured DNA sequences encoding OB receptor, fragments of OB receptor, and analogs of OB receptor which DNA sequences may incorporate codons facilitating transcription and translation of messenger RNA in microbial hosts.. Such manufactured sequences may readily be constructed according to the methods of Alton et al., PCT published application WO 83/04053.

Genomic DNA, such as that of Seq. ID No. 9, encoding the present OB receptors may contain additional non-coding bases, or introns, and such genomic DNAs are obtainable by hybridizing all or part of the cDNA, illustrated in Seq. ID Nos. 2, 4, 6, 8, 11, and 14 to a genomic DNA source, such as a human genomic DNA library. Such genomic DNA will encode functional OB receptor polypeptide; however, use of the cDNAs may be more practicable in that, since only the coding region is involved, recombinant manipulation is facilitated. The 10 intron/exon location of genomic DNA is set forth in Seq. ID No. 9, infra.

Nucleic acid sequences include the incorporation of codons which enhance expression by selected nonmammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences which facilitate construction of cloning and/or expression vectors.

The present invention also provides DNA sequences coding for polypeptide analogs or derivatives of OB receptor which differ from naturally-occurring forms in terms as described above. The leader sequence DNA may be substituted with another leader sequence for ease in expression or for other purposes.

Also, one may prepare antisense nucleic acids against the present DNAs. Such antisense nucleic acids may be useful in modulating the effects of OB receptor protein in vivo. For example, one may prepare an antisense nucleic acid which effectively disables the ability of a cell to produce OB receptor by binding to the nucleic acid which encodes such OB receptor.

DNA sequences of the invention are also suitable materials for use as labeled probes in isolating human genomic DNA encoding OB receptor, as mentioned above, and related proteins as well as cDNA

and genomic DNA sequences of other mammalian species. DNA sequences may also be useful in various alternative methods of protein synthesis (e.g., in insect cells) or, as described infra, in genetic therapy in humans and 5 other mammals. DNA sequences of the invention are expected to be useful in developing transgenic mammalian species which may serve as eucaryotic "hosts" for production of OB receptor and OB receptor products in quantity. See, generally, Palmiter et al., Science 222: 10 809-814 (1983).

Vectors and Host Cells

According to another aspect of the present invention, the DNA sequences described herein which encode OB receptor polypeptides are valuable for the information which they provide concerning the amino acid sequence of the mammalian protein which have heretofore been unavailable. Put another way, DNA sequences provided by the invention are useful in generating new and useful viral and circular plasmid DNA vectors, new and useful transformed and transfected prokaryotic and eucaryotic host cells (including bacterial cells, yeast cells, insect cells, and mammalian cells grown in culture), and new and useful methods for cultured growth 25- of such host cells capable of expression of OB receptor and its related products.

The DNA provided herein (or corresponding RNAs) may also be used for gene therapy for, example, treatment of conditions characterized by the 30 overexpression of OB protein, such as anorexia or cachexia. Alternatively, gene therapy may be used in cases where increased sensitivity to OB protein is desired, such as in cases where an individual has a condition characterized by OB protein receptors 35 defective in ability to bind or retain the binding of OB protein. Currently, vectors suitable for gene therapy

(such as retroviral or adenoviral vectors modified for gene therapy purposes and of purity and pharmaceutical acceptability) may be administered for delivery into the lung, for example. Such vectors may incorporate nucleic acid encoding the present polypeptides for expression in a desired location. Gene therapy may involve more than one gene for a desired protein or different desired proteins.

Alternatively, one may use no vector so as to facilitate relatively stable presence in the host. For example, homologous recombination of a DNA as provided herein or of a suitable transcription or translation control region may facilitate integration into or expression from a host genome. (This may be performed for production purposes as well, e.g., U.S. Patent No. 5,272,071 and WO 91/09955.) The nucleic acid may be placed within a pharmaceutically acceptable carrier to facilitate cellular uptake, such as a lipid solution carrier (e.g., a charged lipid), a liposome, or polypeptide carrier (e.g., polylysine). A review article on gene therapy is Verma, Scientific American, November 1990, pages 68-84 which is herein incorporated by reference.

Thus, the present invention provides for a population of cells expressing an OB receptor of the present OB receptor family. Such cells are suitable for transplantation or implantation into an individual for therapeutic purposes. For example, one may prepare a population of cells to overexpress OB receptor (such as one identified in the Sequence ID's or otherwise denoted herein), or to express a desired form of OB receptor, such as one which is particularly sensitive to OB protein (i.e., a form which has a desired capacity for signal transduction). One may then implant such cells into an individual to increase that individual's sensitivity to OB protein. Such cells may, for example,

be liver cells, bone marrow cells, or cells derived from umbilical cord. Alternatively, one may wish to use overexpressing circulating cells such as blood progenitor cells, T cells or other blood cells. For 5 humans, human cells may be used. Cells may be in the form of tissue. Such cells may be cultured prior to transplantation or implantation. Such OB receptor overexpression, or expression of particularly sensitive forms of OB receptor may be accomplished by, for 10 example, altering the regulatory mechanism for expression of OB receptor, such as using homologous recombination techniques as described supra. Thus, provided is a population of host cells modified so that expression of endogenous OB receptor DNA is enhanced.

15 The cells to be transferred to the recipient may be cultured using one or more factors affecting the growth or proliferation of such cells if appropriate. Hematopoietic factors may be used in culturing hematopoietic cells. Such factors include G-CSF, EPO, 20 MGDF, SCF, Flt-3 ligand, interleukins (e.g., IL1-IL13), GM-CSF, LIF, and analogs and derivatives thereof as available to one skilled in the art.

Nerve cells, such as neurons or glia, may also be used, and these may be cultured with neurotrophic 25 factors such as BDNF, CNTF, GDNF, NT3, or others.

There may be a co-gene therapy involving the transplantation of cells expressing more than one desired protein. For example, cells expressing OB receptor protein may be used in conjunction, 30 simultaneously or in seriatim with cells expressing OB protein.

For gene therapy dosages, one will generally use between one copy and several thousand copies of the present nucleic acid per cell, depending on the vector, 35 the expression system, the age, weight and condition of the recipient and other factors which will be apparent

to those skilled in the art. The cellular delivery of such protein may be designed to last for a selected period of time, such as a period of days, weeks, months or years. At the end of the effective time period, the 5 recipient of such transformed cells may receive another "dose" (e.g., transplantation of cells). Cells may be selected for their lifespan, their time period of expression of the desired protein, or their ability to be reisolated from an individual (i.e., for blood cells, 10 leukaphoresis may be used to retrieve transformed cells using markers present on the cell surface). Vectors may be similarly designed using, for example, viruses which have a known period of expression of DNAs contained therein.

15 The desired cells or vectors may be stored using techniques, such as freezing, available to those in the art.

Thus, the present invention also contemplates a method for administering OB receptor protein to an 20 individual, wherein the source of said OB receptor protein is selected from (i) a population of cells expressing OB receptor protein and (ii) a population of vectors expressing OB receptor protein. Said OB receptor protein may be selected from among those 25 described herein. Said vectors may be virus vectors capable of infecting human cells. Said cells may be selected from among tissue or individual cells. Said individual cells may be selected from among adipocytes, fibroblasts, bone marrow cells, peripheral blood 30 progenitor cells, red blood cells, and white blood cells, including T cells and nerve cells. Said population of cells or vectors may be co-administered with a population of cells or vectors which express OB protein or another desired protein. Said cells or 35 vectors may be stored for use in an individual. Storage may be by freezing

Complexes

In addition to the OB receptor protein as described herein, one may prepare complexes of OB receptor protein and OB protein, analog or derivative.

The OB protein may be selected from those described in PCT publication WO 96/05309, above and hereby incorporated by reference in its entirety. Figure 3 of that publication (Seq. ID No. 4, as cited therein) depicts the full deduced amino acid sequence derived for the human OB gene. The amino acids are numbered from 1 to 167. A signal sequence cleavage site is located after amino acid 21 (Ala) so that the mature protein extends from amino acid 22 (Val) to amino acid 167 (Cys). For the present disclosure, a different numbering is used herein, where the amino acid position 1 is the Valine residue which is at the beginning of the mature protein.

Generally, the OB protein for use will be capable of complexing to the OB protein receptor selected. Thus, one may empirically test the binding capability (to all or part of the extracellular domain of the OB receptor as indicated above) to determine which OB protein forms may be used. Generally, modifications generally applicable as indicated above for OB receptor protein may also be applied here, and that disclosure is incorporated by reference here. As set forth in WO 96 05309, OB protein in its native form, or fragments (such as enzyme cleavage products) or other truncated forms, analogs, and derivatives all retain biological activity. Such forms may be used so long as the form binds to at least a portion of the extracellular domain of the present OB receptor proteins.

An effective amount of an OB protein, analog or derivative thereof may be selected from among

according to the amino acid sequence as presented in PCT  
WO 96/05309, Figure 3 numbered so that the first amino  
acid of the mature protein is number 1:

- (a) the amino acid sequence 1-146,
  - 5 optionally lacking a glutamyl residue at position 28,
    - and further optionally having a methionyl residue at the N-terminus;
- (b) an amino acid sequence of subpart
  - (a) having a different amino acid substituted in one or
    - 10 more of the following positions: 4, 8, 32, 33, 35, 48, 50, 53, 60, 64, 66, 67, 68, 71, 74, 77, 78, 89, 97, 100, 102, 105, 106, 107, 108, 111, 112, 118, 136, 138, 142, and 145;
- (c) a truncated OB protein analog
  - 15 selected from among: (using the numbering of subpart (a) above):
    - (i) amino acids 98-146
    - (ii) amino acids 1-32
    - (iii) amino acids 1-35
    - 20 (iv) amino acids 40-116
    - (v) amino acids 1-99 and 112-146
    - (vi) amino acids 1-99 and 112-146
- having one or more of amino acids 100-111 sequentially placed between amino acids 99 and 112; and,
  - 25- (vii) the truncated OB analog of subpart (i) having one or more of amino acids 100, 102, 105, 106, 107, 108, 111, 112, 118, 136, 138, 142, and 145 substituted with another amino acid;
- 30 (viii) the truncated analog of subpart (ii) having one or more of amino acids 4, 8 and 32 substituted with another amino acid;
- (ix) the truncated analog of subpart (iv) having one or more of amino acids 50, 53, 60, 64, 66, 67, 68, 71, 74, 77, 78, 89, 97, 100, 102,

105, 106, 107, 108, 111 and 112 replaced with  
another amino acid;

(x) the truncated analog of subpart

5 (v) having one or more of amino acids 4, 8, 32, 33,  
35, 48, 50, 53, 60, 64, 66, 67, 68, 71, 74, 77,  
78, 89, 97, 112, 118, 136, 138, 142, and 145  
replaced with another amino acid;

(xi) the truncated analog of subpart

10 (vi) having one or more of amino acids 4, 8, 32, 33,  
35, 48, 50, 53, 60, 64, 66, 67, 68, 71, 74, 77,  
78, 89, 97, 100, 102, 105, 106, 107, 108, 111,  
112, 118, 136, 138, 142, and 145 replaced with  
another amino acid;

15 (xii) the truncated analog of any of  
subparts (i)-(xi) having an N-terminal methionyl  
residue; and

(d) the OB protein or analog derivative  
of any of subparts (a) through (c) comprised of a  
chemical moiety connected to the protein moiety;

20 (e) a derivative of subpart (d) wherein  
said chemical moiety is a water soluble polymer moiety;

(f) a derivative of subpart (e) wherein  
said water soluble polymer moiety is polyethylene  
glycol;

25 (g) A derivative of subpart (f) wherein  
said water soluble polymer moiety is a polyamino acid  
moiety;

(h) a derivative of subpart (g) wherein  
said water soluble polymer moiety is attached at solely  
30 the N-terminus of said protein moiety;

(i) an OB protein, analog or derivative  
of any of subparts (a) through (h) in a pharmaceutically  
acceptable carrier.

OB proteins, analogs and related molecules are  
35 also reported in the following publications; however, no

representation is made with regard to the activity of  
any composition reported:

- 5 U.S. Patent Nos. 5,521,283; 5,532,336;  
5,552,522; 5,552,523; 5,552,524; 5,554,727;  
5,559,208; 5,563,243; 5,563,244; 5,563,245;  
5,567,678; 5,567,803; 5,569,744; 5,569,743  
10 (all assigned to Eli Lilly and Company);  
PCT WO96/23517; WO96/23515; WO96/23514;  
W096/24670; W096/23513; W096/23516;  
W096/23518; W096/23519; W096/23520;  
W096/23815; W096/24670; W096/27385 (all  
15 assigned to Eli Lilly and Company);  
PCT WO96/22308 (assigned to ZymoGenetics);  
PCT WO96/29405 (assigned to Ligand  
15 Pharmaceuticals, Inc.);  
(PCT WO96/31526 (assigned to Amyin  
Pharmaceuticals, Inc.);  
PCT WO96/34885 (assigned to Smithkline Beecham  
PLC);  
20 PCT WO96/35787 (assigned to Chiron);  
(EP 0 725 079 (assigned to Eli Lilly and  
Company);  
EP 0 725 078 (assigned to Eli Lilly and  
Company);  
25 EP 0 736 599 (assigned to Takeda);  
(EP 0 741 187 (assigned to F. Hoffman LaRoche).

To the extent these references provide for  
useful OB proteins or analogs or derivatives thereof, or  
30 associated compositions or methods, such compositions  
and/or methods may be used in conjunction with the  
present OB receptor proteins, such as for co-  
administration (together or separately, in a selected  
dosage schedule) or by complexing compositions to the  
35 present OB protein receptors. With the above provisos,  
these publications are herein incorporated by reference.

Derivatives and Formulations

The present OB protein receptor and/or OB protein (herein the term "protein" is used to include "peptide" and OB protein or receptor analogs, such as those recited infra, unless otherwise indicated) may also be derivatized by the attachment of one or more chemical moieties to the protein moiety. If the present pharmaceutical compositions contain as the active ingredient a complex of OB protein receptor and OB protein, one or both of such proteins may be derivatized. The chemically modified derivatives may be further formulated for intraarterial, intraperitoneal, intramuscular, subcutaneous, intravenous, oral, nasal, pulmonary, topical or other routes of administration. Chemical modification of biologically active proteins has been found to provide additional advantages under certain circumstances, such as increasing the stability and circulation time of the therapeutic protein and decreasing immunogenicity. See U.S. Patent No. 4,179,337, Davis et al., issued December 18, 1979. For a review, see Abuchowski et al., in Enzymes as Drugs. (J.S. Holcerberg and J. Roberts, eds. pp. 367-383 (1891)). A review article describing protein modification and fusion proteins is Francis, Focus on Growth Factors 3: 4-10 (May 1992) (published by Mediscript, Mountview Court, Friern Barnet Lane, London N20, OLD, UK). Preferably, for therapeutic use of the end-product preparation, the chemical moiety for derivatization will be pharmaceutically acceptable. A polymer may be used. One skilled in the art will be able to select the desired polymer based on such considerations as whether the polymer/protein conjugate will be used therapeutically, and if so, the desired dosage, circulation time, resistance to proteolysis, and

other considerations. For the present proteins and peptides, the effectiveness of the derivatization may be ascertained by administering the derivative, in the desired form (i.e., by osmotic pump, or by injection or infusion, or, further formulated for oral, pulmonary or nasal delivery, for example), and observing biological effects as described herein.

The chemical moieties suitable for derivatization may be selected from among various water soluble polymers. The polymer selected should be water soluble so that the protein to which it is attached so that it is miscible in an aqueous environment, such as a physiological environment. The water soluble polymer may be selected from the group consisting of, for example, polyethylene glycol, copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random or non-random copolymers (see *supra* regarding fusion molecules), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols, polystyrene maleate and polyvinyl alcohol. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water.

Fusion proteins may be prepared by attaching polyaminoacids to the OB protein receptor or OB protein (or analog or complex) moiety. For example, the polyamino acid may be a carrier protein which serves to increase the circulation half life of the protein. For the present therapeutic or cosmetic purposes, such polyamino acid should be those which do not create neutralizing antigenic response, or other adverse response. Such polyamino acid may be selected from the

group consisting of serum album (such as human serum albumin), an antibody or portion thereof (such as an antibody constant region, sometimes called "Fc") or other polyamino acids. As indicated below, the location 5 of attachment of the polyamino acid may be at the N-terminus of the OB protein moiety, or other place, and also may be connected by a chemical "linker" moiety to the OB protein.

The polymer may be of any molecular weight, 10 and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 2 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated, 15 molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or 20 lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

The number of polymer molecules so attached may vary, and one skilled in the art will be able to ascertain the effect on function. One may mono- 25 derivatize, or may provide for a di-, tri-, tetra- or some combination of derivatization, with the same or different chemical moieties (e.g., polymers, such as different weights of polyethylene glycals). The proportion of polymer molecules to protein (or peptide) 30 molecules will vary, as will their concentrations in the reaction mixture. In general, the optimum ratio (in terms of efficiency of reaction in that there is no excess unreacted protein or polymer) will be determined by factors such as the desired degree of derivatization 35 (e.g., mono, di-, tri-, etc.), the molecular weight of

the polymer selected, whether the polymer is branched or unbranched, and the reaction conditions.

The chemical moieties should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art. E.g., EP 0 401 384 herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., Exp. Hematol. 20: 1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule (or other chemical moiety) may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residue. Those having a free carboxyl group may include aspartic acid residues, glutamic acid residues, and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecule(s) (or other chemical moiety). Preferred for therapeutic manufacturing purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group. Attachment at residues important for receptor binding should be avoided if receptor binding is desired.

One may specifically desire N-terminally chemically modified protein. Using polyethylene glycol as an illustration of the present compositions, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining

the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective N-terminal chemical modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. See PCT WO 96/11953, herein incorporated by reference. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved. For example, one may selectively N-terminally pegylate the protein by performing the reaction at a pH which allows one to take advantage of the pKa differences between the e-amino group of the lysine residues and that of the a-amino group of the N-terminal residue of the protein. By such selective derivatization, attachment of a polymer to a protein is controlled: the conjugation with the polymer takes place predominantly at the N-terminus of the protein and no significant modification of other reactive groups, such as the lysine side chain amino groups, occurs.

Using reductive alkylation, the polymer may be of the type described above, and should have a single reactive aldehyde for coupling to the protein. Polyethylene glycol propionaldehyde, containing a single reactive aldehyde, may be used.

An N-terminally chemically modified derivative is preferred (over other forms of chemical modification) for ease in production of a therapeutic. N-terminal chemical modification ensures a homogenous product as characterization of the product is simplified relative to di-, tri- or other multi-derivatized products. The use of the above reductive alkylation process for

preparation of an N-terminally chemically modified product is preferred for ease in commercial manufacturing.

In yet another aspect of the present invention, provided are methods of using pharmaceutical compositions of the proteins, and derivatives. Such pharmaceutical compositions may be for administration by injection, or for oral, pulmonary, nasal, transdermal or other forms of administration. In general, comprehended by the invention are pharmaceutical compositions comprising effective amounts of protein or derivative products of the invention together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. Such compositions include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength; additives such as detergents and solubilizing agents (e.g., Tween 80, Polysorbate 80), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol); incorporation of the material into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or into liposomes. See, e.g., PCT WO96/29989, Collins et al., "Stable protein: phospholipid compositions and methods," published October 3, 1996, herein incorporated by reference. Hyaluronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation. Such compositions may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the present proteins and derivatives. See, e.g., Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, PA 18042) pages 1435-1712 which are herein incorporated by reference. The compositions may be prepared in

liquid form, or may be in dried powder, such as lyophilized form. Implantable sustained release formulations are also contemplated, as are transdermal formulations.

5 Specifically contemplated are oral dosage forms of the above derivatized proteins. Protein may be chemically modified so that oral delivery of the derivative is efficacious. Generally, the chemical modification contemplated is the attachment of at least 10 one moiety to the protein (or peptide) molecule itself, where said moiety permits (a) inhibition of proteolysis; and (b) uptake into the blood stream from the stomach or intestine. Also desired is the increase in overall stability of the protein and increase in circulation 15 time in the body. See PCT WO95/21629, Habberfield, "Oral Delivery of Chemically Modified Proteins" (published August 17, 1995) herein incorporated by reference, and U.S. Patent No. 5,574,018, Habberfield et al., "Conjugates of Vitamin B12 and Proteins," issued 20 November 12, 1996, herein incorporated by reference.

Also contemplated herein is pulmonary delivery of the present protein, or derivative thereof. The protein (derivative) is delivered to the lungs of a mammal while inhaling and traverses across the lung 25 epithelial lining to the blood stream. See, PCT WO94/20069, Niven et al., "Pulmonary administration of granulocyte colony stimulating factor," published September 15, 1994, herein incorporated by reference.

Nasal delivery of the protein (or analog or 30 derivative) is also contemplated. Nasal delivery allows the passage of the protein to the blood stream directly after administering the therapeutic product to the nose, without the necessity for deposition of the product in the lung. Formulations for nasal delivery include those 35 with absorption enhancing agents, such as dextran or

cyclodextran. Delivery via transport across other mucous membranes is also contemplated.

Dosages

- 5 One skilled in the art will be able to ascertain effective dosages by administration and observing the desired therapeutic effect. Preferably, the formulation of the molecule or complex in a pharmaceutical composition will be such that between
- 10 about .10 µg/kg/day and 10 mg/kg/day will yield the desired therapeutic effect. The effective dosages may be determined using diagnostic tools over time. For example, a diagnostic for measuring the amount of OB protein or OB receptor protein in the blood (or plasma
- 15 or serum) may first be used to determine endogenous levels of OB protein (or receptor). Such diagnostic tool may be in the form of an antibody assay, such as an antibody sandwich assay. The amount of endogenous OB receptor protein (such as soluble receptor) is
- 20 quantified initially, and a baseline is determined. The therapeutic dosages are determined as the quantification of endogenous and exogenous OB receptor protein (that is, protein, analog or derivative found within the body, either self-produced or administered) is continued over
- 25 the course of therapy. The dosages may therefore vary over the course of therapy, with a relatively high dosage being used initially, until therapeutic benefit is seen, and lower dosages used to maintain the therapeutic benefits.
- 30 During an initial course of therapy of an obese person, dosages may be administered whereby weight loss and concomitant fat tissue decrease increase is achieved. Once sufficient weight loss is achieved, a dosage sufficient to prevent re-gaining weight, yet
- 35 sufficient to maintain desired weight or fat mass may be administered. These dosages can be determined

empirically, as the effects of OB protein are reversible. E.g., Campfield et al., Science 269: 546-549 (1995) at 547. Thus, if a dosage resulting in weight loss is observed when weight loss is not desired, one would administer a lower dose, yet maintain the desired weight.

#### Therapeutic Compositions and Methods

The present OB receptor proteins, alone, or in combination with an OB protein, and nucleic acids may be used for methods of treatment, or for methods of manufacturing medicaments for treatment. Such treatment includes conditions characterized by excessive production of OB protein, wherein the present OB receptors, particularly in soluble form, may be used to complex to and therefore inactivate such excessive OB protein. Or, such OB receptor protein, particularly in soluble form, may act to protect the activity of OB protein. While not wishing to be bound by theory, one may postulate that OB protein receptor agonist activity may be accomplished by a protective effect achieved when OB protein receptor (particularly soluble receptor) is complexed to OB protein. Such effect may prolong the serum half life of OB protein in vivo. Such treatments may be accomplished by preparing soluble receptor (e.g., use of an extracellular domain as described supra) and administering such composition to an individual in need thereof or by preparation of a population of cells containing or expressing such OB receptor, and transplanting such cells into the individual in need thereof.

The present OB receptors may also be used for treatment of those having defective OB receptors. For example, one may treat an individual having defective OB receptors by preparation of a population of cells containing such non-defective OB receptor, and

transplanting such cells into an individual. Or, an individual may have an inadequate number of OB receptors, and cells containing such receptors may be transplanted in order to increase the number of OB receptors available to an individual.

The present OB receptor proteins and related compositions such as OB receptor protein/OB protein complex, provide for weight loss, fat loss, increase in lean mass, increase in insulin sensitivity, increase in overall strength, increase in red blood cells (and oxygenation in the blood), decrease in bone resportion or osteoporosis, decreased or maintained serum cholesterol level, decreased or maintained triglyceride (LDL or VLDL) levels, prevention or reduction in arterial plaque formation, treatment of hypertension, and prevention or reduction of gall stone formation. As body fat composition may be correlated with certain types of cancers, the present compositions may be useful for the prevention or amelioration of certain types of cancers. The present invention also includes methods for manufacture of a medicament for use in conjunction with the cosmetic/therapeutic conditions described herein, containing at least one of the present compositions.

The present compositions and methods may be used in conjunction with other medicaments, such as those useful for the treatment of diabetes (e.g., insulin or analogs thereof, thiazolidinediones or other antihyperglycemic agents, and possibly amylin or antagonists there of), cholesterol and blood pressure lowering medicaments (such as those which reduce blood lipid levels or other cardiovascular medicaments), and activity increasing medicaments (e.g., amphetamines). Appetite suppressants may also be used (such as serotonin modulators and neuropeptide Y antagonists).

(Such administration may be simultaneous or may be in seriatim.

In addition, the present methods may be used in conjunction with surgical procedures, such as cosmetic surgeries designed to alter the overall appearance of a body (e.g., liposuction or laser surgeries designed to reduce body mass, or implant surgeries designed to increase the appearance of body mass). The health benefits of cardiac surgeries, such as bypass surgeries or other surgeries designed to relieve a deleterious condition caused by blockage of blood vessels by fatty deposits, such as arterial plaque, may be increased with concomitant use of the present compositions and methods. Methods to eliminate gall stones, such as ultrasonic or laser methods, may also be used either prior to, during or after a course of the present therapeutic methods. Furthermore, the present methods may be used as an adjunct to surgeries or therapies for broken bones, damaged muscle, or other therapies which would be improved by an increase in lean tissue mass.

In yet another aspect, the present invention provides for methods of manufacture of a medicament for the treatment of obesity, type II diabetes, excess blood lipid, or cholesterol levels, increasing sensitivity to insulin, increasing lean mass, and other conditions as set forth above. Also provided are solely cosmetic treatments for individuals wishing to improve appearance by weight loss, and more specifically, loss of fat deposits, even in the absence of any therapeutic benefit.

#### Diagnostic Compositions and Methods

As indicated supra, polypeptide products of the invention may be "labeled" by association with a detectable marker substance (e.g., radiolabeled with

125I, fluorescent, chemiluminescent, enzyme) to provide reagents useful in detection and quantification of OB receptor (or complexes) in solid tissue and fluid samples such as blood or urine. Nucleic acid products 5 of the invention may also be labeled with detectable markers (such as radiolabels and non-isotopic labels such as biotin) and employed in hybridization processes to locate the human OB receptor gene position and/or the position of any related gene family in a chromosomal 10 map. Nucleic acid sequences which selectively bind the human OB receptor gene are useful for this purpose. They may also be used for identifying human OB receptor gene disorders at the DNA level and used as gene markers for identifying neighboring genes and their disorders. 15 Such nucleic acid sequences may be sued for detection or measurement of OB receptor mRNA level from a biological sample. Contemplated herein are kits containing such labelled materials.

The protein and/or nucleic acids provided 20 herein may also be embodied as part of a kit or article of manufacture. Contemplated is an article of manufacture comprising a packaging material and one or more preparations of the presently provided compositions. Such packaging material will comprise a 25 label indicating that the protein or nucleic acid preparation is useful for detecting and/or quantifying the amount of OB receptor in a biological sample, or OB receptor defects in a biological sample. As such, the kit may optionally include materials to carry out such 30 testing, such as reagents useful for performing DNA or RNA hybridization analysis, or PCR analysis on blood, urine, or tissue samples.

A further embodiment of the invention is 35 selective binding molecules, such as monoclonal antibodies selectively binding OB receptor. The

hybridoma technique described originally by Kohler and Milstein Eur. J. Immunol. 6, 511-519 (1976) has been widely applied to produce hybrid cell lines that secrete high levels of monoclonal antibodies against many specific antigens. Recombinant antibodies, (see Huse et al., Science 246: 1275 (1989)) may also be prepared. Such recombinant antibodies may be further modified, such as by modification of complementarity determining regions to increase or alter affinity, or "humanizing" such antibodies. Such antibodies may be incorporated into a kit for diagnostic purposes, for example. A diagnostic kit may be employed to determine the location and/or amount of OB receptor of an individual. Diagnostic kits may also be used to determine if an individual has receptors which bind OB protein, or those which, to varying degrees, have reduced binding capacity or ability. As stated infra, such antibodies may be prepared using immunogenic portions of an OB receptor protein. Such selective binding molecules may themselves be alternatives to OB protein, and may be formulated for pharmaceutical composition.

Such proteins and/or nucleic acids may be used for tissue distribution assays (for example, as provided in the working example below) or for other assays to determine the location of OB receptor.

The present OB receptor protein family may be used in methods to obtain OB protein analogs, mimetics or small molecules. One would simply prepare a desired OB receptor protein, particularly one with capability of binding to native OB protein, and assay the test molecule, which may be labelled with a detectable label substance, for ability to bind to such receptor. Other parameters, such as affinity, and location of binding, may also be ascertained by methods available to those skilled in the art. For example, one could use portions of the present OB receptors, particularly portions in

the extracellular domain which are necessary for ligand binding, to determine the location of such binding. One could prepare OB receptors which have various truncations or deletions of regions of the extracellular domain which could be used to determine the location of test molecule binding. One could use an OB receptor known to be defective in native OB binding, such as potentially one from an individual having such defective receptors, and use this as the basis for ascertaining OB protein which would be effective to result in desired biological activity (i.e., weight loss, reduction in blood dyslipidemias or lowering of cholesterol levels, reduction in incidence or severity of diabetes). Other uses include solely cosmetic uses for alteration of body appearance, particularly the removal of fat.

The present OB receptor protein or nucleic acids may also be useful to identify substances which "up-regulate" OB protein or receptor. For instance, the temporal expression of OB receptor in vivo may be useful to determine if an administered substance causes an increase or decrease in OB receptor. One may conclude that an increase in OB receptor expression results in modulation of weight or lipid metabolism.

The divergence in the C-terminus may represent OB receptors with different signal transduction abilities. Therefore the different receptor family members may be used for different assays, depending on the type of signal transduction observed. It is thought that at least a portion of the intracellular domain is necessary for signal transduction (see supra).

The following examples are offered to more fully illustrate the invention, but are not to be construed as limiting the scope thereof.

EXAMPLE 1: IDENTIFICATION OF HUMAN OB RECEPTOR PROTEIN

5 Human OB receptor protein DNA was identified in a human liver cDNA library in two steps. The first step used two primers in polymerase chain reaction (PCR) to amplify a selected 300 base pair region from the human liver cDNA library. The second step used the PCR fragment as a probe to screen the human liver cDNA  
10 library. Thirteen clones were obtained, but these were incomplete at the 5' end. A procedure was performed to complete the 5' end to make complete clones. Twelve clones were sequenced. These twelve clones were identified as either "A", "B" or "C" as denoted by the  
15 C-terminus of the predicted amino acid sequence.

Polymerase Chain Reaction.

20 The original PCR primer was based on the 5' end and the 3' end of a 416 base pair sequence having GenBank Database Accession No. T73849. This sequence was selected on the basis of a known motif present in cytokine receptors, "WSXWS".  
25-

The 5' primer had the sequence 73-96 of the 416 bp sequence. The 3' primer had the sequence 337-360 of the 416 bp sequence.

These primers were used to probe a human cDNA liver library (Stratagene). Standard methods were used.

This resulted in a PCR fragment having the sequence 73-360 of the 416 bp fragment.  
30

Hybridization.

35 The 300 bp PCR fragment was used to probe a human liver cDNA library (Stratagene) using standard methods. This second hybridization resulted in 13 positive clones. These were partial clones, incomplete at the 5' end.

Completion of the 5' end.

Rapid Amplification of cDNA End ("RACE", kit, GIBCO/BRL) was used to obtain the full length clones.

5

Sequencing results.

Sequencing revealed the three types of OB receptor DNAs. Of the thirteen clones, 4 clones were the "A" type (Seq. ID Nos. 1 and 2); 1 clone was the "B" type (Seq. ID Nos. 3 and 4) and 4 clones were of the "C" type (Seq. ID Nos. 5 and 6).

As can be seen from the Sequence Identifications (below), OB receptor A is 896 amino acids long, "B" is 904 amino acids long, and "C" is 958 amino acids long. These different OB receptors are identical at amino acid positions 1-891, and diverge almost completely beginning at position 892. The leader sequence is postulated to be, by hydrophobicity analysis, amino acids 1-21(M-A), 1-22(M-F) or 1-28(M-I), with the mature protein beginning at positions 22(F), 23(N) or 29(T). Based on hydrophobicity analysis, the leader sequence is most likely to be at positions 1-21(M through A). Chinese Hamster Ovary Cell ("CHO") cell production of the secreted form of OB receptor protein also produced a protein having amino acid number 22 as the first amino acid of the mature protein. The transmembrane region is likely to begin at either position 840 (A) or 842(L) through position 862(I), 863(S) or 864(H). For OB receptor type "A", the last amino acid is located at position 896 and is a lysine (L). For OB receptor type "B", the last amino acid is located at position 904 and is a glutamine (Q). For OB receptor type "C", the last amino acid is located at position 958 and is glutamic acid (E). For OB receptor protein type "C", the C-terminal region possesses high homology to a known human

transposable element. From nucleotide 2737 through 2947 of the present human OB receptor protein type "C", there is a 98.1% homology with a 211 base section of a human retrotransposable element described in Ono et al., Nucl. Acids Res. 15: 8725-8737 (1987) (bases 520 through 731, SINE-R11, GENBANK accession no. x07417).

EXAMPLE 2: TISSUE DISTRIBUTION

Tissue distribution was ascertained using two methods. The first method involved using the entire type "A" OB receptor. The second method involved using probes which are specific to the C-terminal region of the protein. Since these C terminal regions are divergent, the second method detected the tissue distribution of the different members of the OB receptor family.

The first method used a Northern Blot kit (Clontech), using the entire type A OB receptor DNA as a probe. The second method used PCR with primers specific to the nucleic acids encoding the divergent C terminus of the three types. Standard methods were used.

Table 2 shows the results for the Northern Blot and the PCR methods. The "+" indicates the investigator's subjective determination of the strength of signal. For the Northern Blot analysis, a triple "+++" indicates that a result (a dark "band" on the X-ray film) was seen upon overnight exposure of the film. A double "++" indicates that bands were seen at two weeks of exposure. A single "+" indicates that the bands were seen after three weeks of exposure. In addition, using this method, two molecular weights were observed, one at 4 Kb and one at 6.2 Kb. Although distribution was ubiquitous, the strongest signals were seen for ovary, heart and liver. For the PCR analysis, OB receptor "A" was seen in all tissue types tested (prostate, ovary, small intestine, heart, lung, liver

and skeletal muscle), type "B" was seen only in lung and liver, and type "C" was seen in ovary, heart, lung and liver.

5

Table 2

Tissue Distribution of the Novel OB Receptor

|                            | Northern Blot |        | PCR |   |   |
|----------------------------|---------------|--------|-----|---|---|
|                            | 4 Kb          | 6.2 Kb | A   | B | C |
| Spleen                     | -             | +      |     |   |   |
| Thymus                     | -             | +      |     |   |   |
| Prostate                   | -             | +      | +   | - | - |
| Testis                     | -             | +      |     |   |   |
| Ovary                      | -             | +++    | +   | - | + |
| Small Intestine            | -             | ++     | +   | - | - |
| Colon                      | -             | -      |     |   |   |
| Peripheral blood Leukocyte | -             | -      |     |   |   |
| Heart                      | -             | +++    | +   | - | + |
| Brain                      | -             | -      |     |   |   |
| Placenta                   | -             | +      |     |   |   |
| Lung                       | +             | ++     | +   | + | + |
| Liver                      | +++           | +++    | +   | + | + |
| Skeletal Muscle            | -             | ++     | +   | - | - |
| Kidney                     | -             | ++     |     |   |   |
| Pancreas                   | -             | +      |     |   |   |

10

EXAMPLE 3: IDENTIFICATION OF HUMAN OB RECEPTOR GENOMIC DNA AND CHROMOSOME LOCALIZATION; IDENTIFICATION OF HUMAN OB RECEPTOR "D"

15

The full length human OB receptor genomic DNA was also prepared. OB receptor "A" cDNA, in its entirety, was used as a probe against a human genomic DNA library, using materials and methods from a commercially available kit (Genome Systems, using a human genomic library in a P1 vector). A single

20

positive clone was detected. There are introns located at (with respect to OB receptor "A" DNA) base pair number: 559, 1059, 1350, 1667, 1817, 1937, 2060, 2277, 2460, 2662, and 2738.

5 The human OB receptor gene was localized to human chromosome 1P31 by FISH analysis (Genome Systems). Human chromosome 1 is thought to correspond to mouse chromosome 4C7, which is presumed to be the location of the *db* locus.

10 A further chromosomal sequence was isolated. This chromosomal DNA sequence was isolated from a human genomic library as described above. This chromosomal sequence encodes what is here denominated human OB receptor "D", and the encoded amino acid sequence is set forth in SEQ. ID No. 7. A cDNA encoding this amino acid sequence is set forth in SEQ. ID No. 8. The chromosomal DNA intron/exon junction map is set forth as SEQ. ID No. 9.

As with forms "A", "B", and "C", for the present form "D" OB receptor protein, the first amino acid of the mature protein is likely (using hydrophobicity analysis) to begin at position 22 (F), 23 (N) or 29 (T). The last amino acid of the protein is at position 1165 and is a valine residue. As with the other forms, the extracellular domain extends from position 22 (F), 23 (N) or 29 (T) to position 839 (D) or 841 (G). The transmembrane domain appears to begin at position 840 (A) or 842 (L). The end of the transmembrane domain appears to be located at position 862 (I), 863 (S) or 864 (H). The C-terminal region, beyond the transmembrane region, is likely to be involved in signal transduction, and is located at position 863 (S), 864 (H) or 865 (Q) through position 1165 (V).

35 The present OB receptor form "D" is identical to that published by Tartaglia et al, Cell 83: 1263-1271.

(December 29, 1995) with the exception of a single amino acid change at amino acid position 976 (nucleotide codon begining at position 3022). The present type "D" amino acid at position 976 is aspartic acid, and the published 5 amino acid corresponding to the same position is alanine. This is a non-conservative substitution, see infra, and since the location of the substitution is within a region thought important for signal trans-duction, this change could affect the function of the 10 molecule.

**EXAMPLE 4: PREPARATION OF SOLUBLE OB RECEPTOR**

Three forms of soluble human OB receptor have 15 been prepared:

1. Leader + Extracellular Domain (Seq. ID Nos. 10 and 11): A recombinant form of the soluble human OB receptor was prepared. This form encompasses, in the immature protein, the leader sequence and the 20 extracellular domain (amino acids 1-839). The mature protein would have the leader sequence deleted, and the first amino acid of the mature recombinant soluble human OB receptor would be 22 (F), 23 (N) or 29 (T). This protein was expressed as described below.

2. Leader + Extracellular Domain + C-terminal FLAG (Seq. ID No. 12): A second form of the recombinant soluble human OB receptor was also prepared. This form had a "FLAG" tag located at the "C" terminus 30 of the protein. The "FLAG" peptide is a useful research tool as it allows one to follow the protein using an antibody which recognizes the "FLAG" peptide. Such reagents are commercially available (IBI, New Haven, CT). This protein was expressed as described below.

3. Native Splice Variant (Seq. ID Nos. 35 13 and 14): This form is believed to be the recombinant form of a naturally occurring secreted,

soluble human OB receptor. This form has most of the amino acids found in the extracellular domain (amino acids 22-798), and a unique 6 amino acid sequence at the carboxyl terminus. Beginning at amino acid position 799 of Seq. ID No. 13, the amino acid sequence of this native splice variant human OB receptor protein is "G K F T I L."

EXAMPLE 5: PREPARATION OF EXPRESSION VECTORS

10

Recombinant human OB receptor expression vectors have been prepared for expression in mammalian cells. As indicated above, expression may also be in non-mammalian cells, such as bacterial cells. The type "A" cDNA (Seq. ID No. 2) was placed into a commercially available mammalian vector (pCEP4, Invitrogen) for expression in mammalian cells, including the commercially available human embryonic kidney cell line, "293".

Recombinant human OB receptor expression vectors have been prepared for expression of recombinant soluble OB receptor, consisting of the leader sequence and the extracellular domain (Seq. ID Nos. 10 and 11), using the same system as above (the commercially available mammalian vector pCEP4, and "293" cells). This recombinant soluble human OB receptor was also expressed in CHO cells in a similar way.

The "FLAG-tagged" form (Seq. ID No. 12) of the recombinant soluble human OB receptor, and the "D" form (Seq. ID No. 7) were also expressed in "293" cells in a similar fashion as above.

Detection of desired protein was accomplished using BIACORE (Pharmacia) analysis. This analysis is analogous to that described in Bartley et al., Nature 368: 558-560 (1994).

Essentially, the BIACORE machine measures affinity interactions between two proteins. In this

case, the OB protein was immobilized on the machine, and conditioned media from cell lines expressing the OB receptor was added to the machine. Any receptor protein present in the conditioned media bound to the OB protein surface. The BIACORE machine gave a read-out indicating that receptor protein was being expressed. For recombinant soluble receptor (Seq. ID No. 10) expression in "293" cells, the read-out was 191.0 relative to a baseline readout of 0. For recombinant soluble receptor (Seq. ID No. 10) expression in CHO cells, the read-out was 150.9 relative to a baseline readout of 0. For recombinant soluble receptor with a C-terminal FLAG-tag (Seq. ID. No. 12), the read-out was 172.0 relative to a baseline of 0.

For expression in bacterial cells, one would typically eliminate that portion encoding the leader sequence (e.g., potentially amino acids 1-21, 1-22 or 1-28). One may add an additional methionyl at the N-terminus for bacterial expression. Additionally, one may substitute the native leader sequence with a different leader sequence, or other sequence for cleavage for ease of expression.

EXAMPLE 6: DEMONSTRATION OF SIGNAL TRANSDUCTION

This example demonstrates that the "D" form is active to produce a signal within a cell, whereas in the same cell type, the "A" form does not. The signal transduction assay was performed by the use of "293" cells transiently expressing either the "A" or the "D" form (see above for preparation of the "293" expression clones). Phosphorylation of molecules predicted to be involved in signal transduction within the cell was examined upon OB protein binding to the OB receptor protein tested. The results demonstrate that upon binding of OB protein to the extracellular domain, the

"D" form of the present OB protein receptor transduces a signal sufficient to initiate phosphorylation of signalling molecules.

5 Methods

1. OB receptor molecules. As indicated above, the "A" form (Seq. ID No. 1) and the "D" form (Seq. ID. No. 7) were studied.

10 2. Expression system. The pCEP 4 system (as described above) having inserted DNA encoding the "A" form (Seq. ID No. 2) or the "D" form (Seq. ID No. 8) was used to transfet "293" cells. These cells did not allow for the pCEP4 vector to integrate into the genome, so such expression was transient. Non-recombinant 15 (mock-transfected) cells were also prepared as controls.

20 3. Detection of phosphorylation. Mock transfected cells and cells expressing the "A" form or the "D" form were analyzed. Prior to treatment the cells were serum-starved by incubation in media with 0.5% serum for 16 hours prior to the treatments. The cells were treated with the OB protein (10 mg/ml) for 15 minutes at 37°C, after which the cells were lysed in modified NP40 buffer (50 mM Tris, pH 8.0, 150 mM sodium chloride, 1% NP40, 10 mg/ml aprotinin, 5mM EDTA, 200 mM 25 sodium orthovanadate). Phosphotyrosine containing proteins were immunoprecipitated (Anti-phosphotyrosine antibody 4G10, UBI, Lake Placid, NY), and separated by SDS polyacrylamide gel electrophoresis. After electrophoresis and electroblotting to membranes the 30 immunoprecipitates were probed with antibodies to various signal transduction molecules. Antibodies to STATs, JAKs and ERKs were purchased from Santa Cruz Biotechnology Inc. Immune complexes were detected by horseradish peroxidase conjugated secondary reagents 35 using chemiluminescence as described by the manufacturer (ECL, Amersham). As a positive control, 32D cells were

treated with IL-3, which is known to activate by tyrosine phosphorylation most of the molecules being analyzed.

4. Results. Results are presented in Table 5, below. As can be seen, only the "D" form was able to respond to either mouse or human OB protein as detected by phosphorylation of JAK and STAT molecules. A "+" designation indicates signal was detected, a "-" designation means that no signal was observed.

10

TABLE 3

| Signal /AB# | 293 Alone | 293/D hrOB* | 293/D mrOB** | 293/A hrOB# | 293/A mrOB## | 32D IL-3 |
|-------------|-----------|-------------|--------------|-------------|--------------|----------|
| STAT1       | -         | +           |              |             |              |          |
| STAT3       | -         | +           | +            | -           | -            | +        |
| STAT5       | -         | +           | +            |             |              | +        |
| JAK1        | -         | +           | +            | -           | -            | +        |
| JAK2        | -         | +           | +            | -           | -            | +        |
| JAK3        | -         | -           | -            |             |              | -        |
| TYK2        | -         | +           | +            |             |              | -        |
| ERKs<br>1,2 | -         | -           | -            | -           | -            | +        |

# Antibody detection target

\* 293 cells expressing receptor form "D", treated with  
15 recombinant human OB

\*\* 293 cells expressing receptor form "D" treated with  
recombinant murine OB

# 293 cells expressing receptor form "A" treated with  
recombinant human OB

20 ## 293 cells expressing receptor form "A" treated with  
recombinant murine OB

The "D" form is capable of initiating signalling through the JAK/STAT pathways in 293 cells, whereas the "A" form cannot.

EXAMPLE 7: USE OF SOLUBLE OB RECEPTOR AS A THERAPEUTIC

This example demonstrates that soluble OB receptor protein acts to protect the activity of OB protein. Below, soluble OB receptor and/or OB protein was delivered to a mammal via "gene transplant" -- that is, via bone marrow cells engineered to express the desired DNAs. When soluble OB receptor combined with OB protein was delivered, the animals lost more weight than delivery of OB protein alone. This demonstrates the protective activity of OB receptor protein.

While not wishing to be bound by theory, one explanation of the mode of action is that soluble OB receptor protein acts to protect the OB protein in serum from agents or conditions which could diminish its activity. The protective action appears to increase circulating half-life of the protein. As such, the present example demonstrates that OB receptor either alone, or administered as a complex with OB protein (or analog or derivative thereof) could act as a therapeutic agent.

Materials and methods:

25. 1. Preparation of recombinant ob retroviral vector Packaging Cells.

Use of murine ob cDNA. Full length wild-type murine ob cDNA was amplified by the PCR using synthetic oligonucleotides designed from the published sequence 30 Zhang et al., Nature 372: 425-432 (1994). Linkers (An Eco RI linker and a Bgl II linker) were used to facilitate subcloning.

Use of soluble recombinant human OB receptor cDNA. Methods similar to those above were used. A 35 construct containing the recombinant human soluble receptor of Seq. ID No. 10 was used, and modified with

{ linkers to facilitate cloning (i.e., the addition of a  
Bgl II restriction endonuclease recognition site).

Placement of desired cDNA into vector. PCR products were digested with EcoRI and BglII and cloned into similarly-digested parental vector (pMSCV2.1) under the transcriptional control of the viral LTR promoter. The parental MSCV vector (supplied by R. Hawley, University of Toronto, Canada) was derived from MESV (murine embryonic stem cell virus) and contains a neomycin phosphotransferase resistance (neor) gene driven by an internal mouse phosphoglycerate kinase (PGK) promoter, as described. Hawley, et al, J. Exp. Med. 176: 1149 -1163 (1992). The parental plasmid pMSCV2.1 and pMSCV-OB were independently electroporated into the GP+E-86 packaging cell line (supplied by Dr. A. Bank, Columbia University, NY) Markowitz et al., J. Virol. 62:1120-1124 (1988). Transient supernatants were harvested from electroporated populations and used to infect tunicamycin treated parental GP+E-86 cells.

Tunicamycin treatment relieves the block to superinfection of the parental packaging cells. G418 (0.78 mg/mL, 67% active, GIBCO Laboratories, Life Technologies, Inc., Grand Island, NY) resistant clones were selected from each infected population and titered by infection of NIH3T3 cells. Clones with the highest G418 resistant titer were expanded and frozen as aliquots. Each bone marrow infection and transplantation experiment used aliquots from the same passage of frozen viral packaging cells. Both the parental and ob packaging cell lines were tested for the presence of, and found to be free from, replication competent virus using a sensitive marker rescue assay. Moore, et al., (1993) in: Gene Targeting: A Practical Approach, Joyner, Ed. (Oxford University Press, New York, NY).

## 2. Production of Retroviral Supernatants.

Recombinant virus-producing packaging cell lines were grown in 175cm<sup>2</sup> tissue culture flasks in Iscove's Modified Dulbecco's Medium (IMDM) (GIBCO), 10% (v/v) FBS, at 37°C. Sub-confluent (approximately 60%) monolayers of cells were fed with fresh medium 24h prior to harvest of virus-containing supernatants. Viral supernatants were removed from packaging cell lines by aspiration, sterile filtered (0.45mM) and added directly to bone marrow cultures. Fresh aliquots of frozen packaging cell lines were thawed for use in each experiment.

## 3. Bone Marrow Infection and Transplantation.

Eight to 12-week old female C57BL/6J (+/+) or (ob/ob) mice were used as bone marrow donors and recipients. All mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed under specific pathogen-free conditions in a vivarium in accordance with governmental regulations and institutional guidelines.

Bone marrow cells were harvested from femurs and tibias of donor mice 4 days post 5-fluorouracil (5-FU, Sigma Chemical Co., St. Louis, MO) treatment (150 mg/kg i.v.). Bone marrow cells ( $6 \times 10^5$ /mL) were incubated in 150mm tissue culture dishes (30mL/dish) containing fresh viral supernatant (as described above), 15% FBS, 6 mg/mL polybrene (Sigma), 0.1% bovine serum albumin (BSA, Fraction V, Sigma), 2.5 ng/mL recombinant mouse IL-3 (rmIL-3), 100 ng/mL each of recombinant human IL-6 (rhIL-6), recombinant human IL-11 (rhIL-11), and recombinant rat SCF (rrSCF). All growth factors were produced by Amgen, Inc. (Thousand Oaks, CA). Culture media were replaced daily for 3 days with fresh virus-containing supernatant and growth factors.

At the end of the infection period, total non-adherent and adherent cells were washed and resuspended in 1% BSA-saline and transplanted into  $\gamma$ -irradiated (12

Gy, Cs<sup>137</sup>) mice. Each animal was transplanted with 2.5 X 10<sup>6</sup> syngeneic cells. There were approximately 10 animals per cohort.

4. Analysis of OB protein expression in transfected cells and transplanted animals. For transfected bone marrow cells, Western analysis was performed. Vector packaging cell supernatant was resolved by SDS-PAGE (16% acrylamide), then transferred to Hybond-ECL (Amersham, Arlington Heights, IL). The filter was incubated with affinity-purified rabbit a-mouse OB protein polyclonal antibody (1mg/mL) in T-TBS buffer (20mM Tris-chloride, pH7.6, 137mM NaCl, 0.1% Tween20) at room temperature for 45 min. Horseradish peroxidase (HRP)-conjugated donkey a-rabbit IgG (Amersham) was diluted in T-TBS (1:2500) and incubated with the filter at room temperature for 45 min. Enhanced chemiluminescence (ECL, Amersham) detection was performed as recommended by the manufacturer.

For transplanted animals, serum was analyzed. Animals were bled retroorbitally, under isofluorane anesthesia. Serum from transplanted ob/ob animals was resolved by SDS-PAGE (4-20% acrylamide) under non-reducing and reducing conditions, then transferred to Trans-Blot (Bio-Rad Laboratories, Hercules, CA) membranes. The membranes were incubated for 2 hours at room temperature with HRP-conjugated rabbit a-mouse OB protein antibody (0.125mg/mL) in T-TBS buffer containing 5% fetal bovine serum and 1% bovine serum albumin. Bound OB protein was detected by ECL (Amersham), performed as recommended by the manufacturer.

For quantitation of soluble OB protein levels, serum from transplanted animals was subjected to ELISA analysis. Briefly, affinity-purified rabbit a-OB protein polyclonal antibody was coated onto 96-well plates. Standards (purified recombinant OB protein

monomer, Pelleymounter et al., Science 269: 540-543 (1995) and experimental samples were added, and the plates were incubated at room temperature. The plates were washed twice and affinity-purified rabbit a-OB protein antibody conjugated to horseradish peroxidase was added. Following incubation at room temperature, the plates were washed four times with TNE-Tween20. TMB/peroxide substrate was added and the color reaction was read at 450nm in a Molecular Devices plate reader. OB protein concentrations in sera were estimated by comparison to a standard curve prepared from internal standards. OB protein levels were reliably measured in samples containing >160 pg/mL.

5. Body Weight and Food Intake. Mice were offered pelletized rodent chow (PMI Feeds, Inc., St. Louis, MO) ad libitum. The body weight of individual animals was measured daily for the first two months of analysis, and weekly thereafter. Food consumption was measured daily on selected groups of individually-housed animals.

### Results

Results are presented in Tables 4 and 5 below. Administration of OB protein receptor increased the effectiveness of OB protein. This may have been accomplished via an increased circulation time of OB protein in the presence of OB protein receptor.

As can be seen in the Table, animals administered a combination of OB protein and OB protein receptor (via genetic therapy) had a greater weight loss after 28 days than either composition alone. The Table presents the results of two experiments ("\_\_\_\_/\_\_\_\_"). As can be seen, use of the OB protein alone at day 40 resulted in animals with 87.5% and 72.2% of the starting weight. Using OB receptor in combination with OB protein, however, resulted in animals with 68% and

53.6% of the starting weight. Use of the receptor alone appeared to have little effect, if any.

(TABLE 4)

| Treatment           | Weight (g)<br>decrease at<br>day 28 (ave) | % starting<br>weight<br>(ave)<br>day 28 | % starting<br>weight<br>(ave)<br>day 40 |
|---------------------|---|---|---|
| OB alone*           | 6.3/12.7                                  | 87.9/75.3                               | 87.5/72.2                               |
| Receptor**<br>alone | [1.4]/[0.3]                               | 103/100.6                               | 104.2/101.7                             |
| OB +<br>Receptor*** | 12.6/16.8                                 | 76.3/67.5                               | 68/53.6                                 |

\* 50% bone marrow cells transfected with OB protein cDNA as described above, and 50% bone marrow cells without genetic alteration

10 \*\* 50% bone marrow cells transfected with OB receptor protein cDNA as described above, and 50% bone marrow cells without genetic alteration

\*\*\* 50% bone marrow cells transfected with OB protein cDNA as described above, and 50% bone marrow cells transfected with OB receptor protein cDNA as described 15 above.

Table 5, below, contains results of the OB levels found in the serum from animals administered OB protein alone, or administered OB protein in combination with OB protein receptor (via the "gene therapy" method 20 of this example). The data reflect nanograms of OB protein per milliliter of serum, plus or minus the standard error of the mean.

TABLE 5

| Treatment           | Experiment #1† | Experiment #2‡‡ |
|---------------------|----------------|-----------------|
| OB alone*           | 2.93 +/- 0.77  | 9.74 +/- 1.02   |
| Receptor**<br>alone | 0.08 +/- 0.05  | 0.12 +/- 0.07   |
| OB +<br>Receptor*** | 12.11 +/- 1.90 | 15.18 +/- 2.52  |

- \* 50% bone marrow cells transfected with OB protein  
 5 CDNA as described above, and 50% bone marrow cells without genetic alteration  
 \*\* 50% bone marrow cells transfected with OB receptor protein cDNA as described above, and 50% bone marrow cells without genetic alteration  
 10 \*\*\* 50% bone marrow cells transfected with OB protein cDNA as described above, and 50% bone marrow cells transfected with OB receptor protein cDNA as described above.  
 † Experiment #1 was conducted as described above,  
 15 with OB protein serum levels measured after 38 days.  
 ‡‡ Experiment #2 was also conducted as described above, with OB protein serum levels measured after 24 days.

The data demonstrate the protective effects of OB receptor. As can be seen, in the presence of OB receptor, OB protein has a higher accumulation in the serum. The degree of accumulation is observed to increase inversely with the levels of OB protein in the serum. In Experiment #1 (with a base OB protein level of about 2.93 ng/ml), the OB protein serum level increased about 400% with the addition of receptor, where in Experiment #2 (with a base of about 9.74), the OB protein serum level increased by about 25%.  
 20 OB receptor administered either alone or in association with OB protein (or analogs or derivatives

thereof) may serve to increase the circulation time of OB protein, and therefore enhance the therapeutic efficacy of either exogenous or endogenous OB protein.

5      EXAMPLE 8: PREPARATION OF SELECTIVE BINDING MOLECULES

Animals were immunized for the preparation of polyclonal antibodies using the following peptides (with respect to the numbering of the amino acids for OB receptor A, Seq. ID No. 1): 54-64; 91-100; 310-325; 10 397-406; 482-496; 874-885; and, with respect to amino acids of OB receptor "C" (Seq. ID No. 5), 910-929. Some of the polyclonal antibodies prepared (in rabbits) were tested for ability to bind to recombinant human OB receptor protein. The polyclonal antibody prepared against amino acids 54-64 was found to have the highest affinity for recombinant human OB receptor protein. The polyclonal antibody prepared against amino acids 397-406 was also found to bind to recombinant human OB receptor protein. The polyclonal antibody prepared against amino acids 91-100 was found to slightly bind to recombinant human OB receptor protein. The polyclonal antibody prepared against amino acids 874-885 was found not to bind to recombinant human OB receptor protein.

An additional study was performed which demonstrates the expression and purification of the extracellular domain of the OB receptor protein in CHO cells, and antibodies which recognize this OB protein receptor extracellular domain.

The extracellular domain of the human OB receptor protein was expressed as a secreted, soluble protein in CHO cells as previously described supra. Individual cell lines were isolated and grown in increasing amounts of methotrexate to increase selection/expression of the recombinant receptor protein 30 (100, 200 or 500 micrograms methotrexate per ml of media). Conditioned media from the CHO cell lines was

- collected, and the proteins in the conditioned media were fractionated by SDS-PAGE. The OB receptor extracellular domain migrated as a broad band with an apparent size range of about 140 kDa to about 200 kDa.
- 5 The OB receptor protein extracellular domain was detected by Western Blot analysis using polyclonal antibodies prepared against a portion of the extracellular domain of the OB receptor protein. The unfolded, bacterially expressed protein was used as an antigen to generate antisera in rabbits. The identified OB receptor extracellular domain was purified by affinity chromatography. The purified protein was sequenced at the amino terminus to confirm that it was the OB receptor and also to determine the start of the mature protein (after signal peptide cleavage) as expressed in CHO cells. It was found that amino acid no. 22 (according to the amino acid sequence numbering of Seq. ID No. 1, *infra*), was the first amino acid of the mature protein as expressed in CHO cells.
- 10 20 Other immunogenic peptides may be used. Polyclonal, monospecific polyclonal, monoclonal, antibody fragments, and recombinant antibodies may be prepared using methods available to those skilled in the art.
- 15 25 One may further use recombinant techniques or peptide synthesis methods to alter the character of such selective binding molecules. This may be accomplished by preparing recombinant antibodies having altered complementarity determining regions (sometimes referred to in the art as "CDR's") to, for example "humanize" the antibodies by using human Fc (constant) regions. Other types of recombinant antibodies, for example, those having CDR's altered to enhance affinity or selectivity to one or more members of the OB receptor family, may be 30 35 prepared and used using methods available to those

skilled in the art. See Winter et al., Nature 349: 293-299 (1991).

The present OB receptor protein may be used as an assay to screen for desired selective binding molecules. Such assay may be based on binding capability, or biological activity, or, other means of detecting signal transduction. For example, if one were to prepare a series of modified antibodies, one could test them for affinity (i.e., binding strength) against the target OB receptor.

The selective binding molecules may be useful for diagnostic purposes, such as tissue distribution analysis, or to diagnose the relative affinity of an individual's OB receptors for such selective binding molecule to determine the functionality of an individual's OB receptor during a course of therapy. Selective binding molecules may be alternative therapeutic or cosmetic products to OB protein.

20 EXAMPLE 9: GENE THERAPY

One may deliver the present OB receptor protein via gene therapy, as described infra.

One may envision, using materials and methods available to those skilled in the art and provided herein, using T-cells as an agent carrying DNA expressing OB receptor for gene therapy. An individual would have T-cells selected using CD34+ selection and a magnetic microparticles selection device. Such cells would be transfected with the desired DNA, or the regulation of the desired coding region may be altered using homologous recombination or other in situ techniques. The transduced cells could be selected empirically, using means to detect the desired protein, or a marker may be included which permits indirect detection (i.e., a selectable marker as is known in the

{ art). Optionally, such cells could be expanded, for example, using one or more growth factors such as SCF or an interleukin, and such cells could be stored for future use. In such a way, the procedure would only  
5 have to be accomplished once or infrequently in an individual's lifetime, for later transfer into the individual. The cells would be re-planted into the individual, and the individual would be monitored for desired therapeutic effect, such as weight  
10 loss/maintenance of weight, diabetes recurrence, blood lipid levels, or other conditions.

Illustrative Nucleic Acid and Amino Acid Sequences

The below amino acid and DNA sequences are  
15 those to which reference has been made. An asterick ("\*") indicates the position of a stop codon.

Human OB Receptor "A" Amino Acid Sequence (Seq. ID No. 1 (Amino Acid, single letter abbreviation)):

1 MICQKFCVVL LHWEFIYVIT AFNLSSYPITP WRFKLSCMPP NSTYDYFLLP  
5 51 AGLSKNTSNS NGHYETAVEP KFNSSGTHFS NLSKTTFHCC FRSEQDRNCS  
10 101 LCADNIEGKT FVSTVNSLVF QQIDANWNIQ CWLKGDALKF ICYVESLFKN  
15 151 LFRNYNYKVH LLYVLPEVLE DSPLVPQKGS FQMVHCNCV HECCECLVPV  
20 201 PTAKLNDTLL MCLKITSGGV IFQSPLMSVQ PINMVKPDPPI LGLHMEITDD  
25 251 GNLKISWSSP PLVPFPLQYQ VKYSENSTTV IREADKIVSA TSLLVDSILP  
30 301 GSSYEVQVRG KRLDGPGIWS DWSTPRVFDT QDVIYFPPKI LTSVGSNVSF  
35 351 HCIYKKENKI VPSKEIVWWM NLAEKIPQSQ YDVVSDHVS K VTFFNLNETK  
40 401 PRGKFTYDAV YCCNEHECHH RYAELYVIDV NINISCETDG YLTKMTCRWS  
45 451 TSTIQSLAES TLQLRYHRSS LYCSDIPSIIH PISEPKDCYL QSDGFYECIF  
50 501 QPIFLLSGYT MWIRINHSLG SLDSPPTCVL PDSVVVKPLPP SSVKAEITIN  
55 551 IGLLKISWEK PVFPENNQF QIRYGLSGKE VQWKMYEVYD AKSKSVSLPV  
60 601 PDLCAVYAVQ VRCKRLDGLG YWSNWSNPAY TVVMDIKVPM RGPEFWRIIN  
65 651 GDTMKKEKNV TLLWKPLMKN DSLCSVQRYV INHHTSCNGT WSEDVGNHTK  
70 701 FTFLWTEQAH TVTVLAINSI GASVANFNLT FSWPMSKVNI VQSL SAYPLN  
75 751 SSCVIVSWIL PSDYKLMYF IIEWKLNED GEIKWLRISS SVKKYYIHDH  
80 801 FIPIEKYQFS LYPIFMEGVG KPKIINSFTQ DDIEKHQSDA GLYVIVPVII  
85 851 SSSILLGTL LISHQRMKKL FWEDVPNPKN CSWAQGLNFQ KRTDIL\*SLI  
90 901 MITTDEPNVP TSQQSIEY\*K IFTF\*RRGAN LKKIQLNF\*E LTYGGLC\*FR  
95 951 T\*NRCVNLGS KCRFESSLVDV \*L

Human OB Receptor "A" DNA Sequence (Seq. ID No. 2 (DNA)):

1 CCGCCGCCAT CTCTGCCTTC GGTCGAGTTG GACCCCCGGA TCAAGGTGTA  
5 51 CTTCTCTGAA GTAAGATGAT TTGTCAAAAA TTCTGTGTGG TTTTGTACA  
10 101 TTGGGAATT ATTATGTGA TAACTGCCTT TAAACTGTCA TATCCAATTA  
15 151 CTCCTTGGAG ATTTAAGTTG TCTTGCATGC CACCAAATTC AACCTATGAC  
20 201 TACTTCCTT TGCGCTGCTGG ACTCTCAAAG AATACTCAA ATTCAATGG  
25 251 ACATTATGAG ACAGCTGTTG AACCTAAGTT TAATTCAAGT GGTACTCACT  
30 301 TTTCTAACTT ATCCAAAACA ACTTTCCACT GTTGCTTCG GAGTGAGCAA  
35 351 GATAGAAACT GCTCCTTATG TGCAGACAAC ATTGAAGGAA AGACATTGT  
40 401 TTCAACAGTA AATTCTTAG TTTTCAACA AATAGATGCA AACTGGAACA  
45 451 TACAGTGCTG GCTAAAAGGA GACTTAAAT TATTCATCTG TTATGTGGAG  
50 501 TCATTATTTA AGAATCTATT CAGGAATTAT AACTATAAGG TCCATCTTT  
55 551 ATATGTTCTG CCTGAAGTGT TAGAAGATTAC ACCTCTGGTT CCCCAAAAG  
60 601 GCAGTTTCAG GATGGTTCAC TGCAATTGCA GTGTCATGA ATGTTGTGAA  
65 651 TGTCTTGTGC CTGTGCCAAC AGCCAAACTC AACGACACTC TCCTTATGTG  
70 701 TTTGAAAATC ACATCTGGTG GAGTAATTTC CCAGTCACCT CTAATGTCAG  
75 751 TTCAGCCCCAT AAATATGGTG AAGCCTGATC CACCATTAGG TTTGCATATG  
80 801 GAAATCACAG ATGATGGTAA TTTAAAGATT TCTTGGTCCA GCCCACCATT  
85 851 GGTACCATT CCACTTCAAT ATCAAGTGAA ATATTCAAGAG AATTCTACAA  
90 901 CAGTTATCAG AGAAGCTGAC AAGATTGTCT CAGCTACATC CCTGCTAGTA  
95 951 GACAGTATAAC TTCCCTGGGTC TTCTGTGAG GTTCAGGTGA GGGGCAAGAG  
100 1001 ACTGGATGGC CCAGGAATCT GGAGTGACTG GAGTACTCCT CGTGTCTTA  
105 1051 CCACACAAAGA TGTCAATAC TTTCCACCTA AAATTCTGAC AAGTGTGGG  
110 1101 TCTAATGTTT CTTTCACTG CATCTATAAG AAGGAAAACA AGATTGTTCC  
115 1151 CTCAAAAGAG ATTGTTGGT GGATGAATT AGCTGAGAAA ATTCCCTCAA  
120 1201 GCCAGTATGA TGTTGTGAGT GATCATGTTA GCAAAGTTAC TTTTTCAAT  
125 1251 CTGAATGAAA CCAAACCTCG AGGAAAGTTT ACCTATGATG CAGTGTACTG

1301 CTGCAATGAA CATGAATGCC ATCATCGCTA TGCTGAATT TATGTGATTG  
1351 ATGTCAATAT CAATATCTCA TGTGAAACTG ATGGGTACTT AACTAAAATG  
5 1401 ACTTGCAGAT GGTCAACCAG TACAATCCAG TCACTTGCGG AAAGCACTTT  
1451 GCAATTGAGG TATCATAGGA GCAGCCTTA CTGTTCTGAT ATTCCATCTA  
10 1501 TTCATCCCCT ATCTGAGCCC AAAGATTGCT ATTTGCAGAG TGATGGTTT  
1551 TATGAATGCA TTTTCCAGCC AATCTTCCTA TTATCTGGCT ACACAATGTG  
1601 GATTAGGATC AATCACTCTC TAGGTTCACT TGACTCTCCA CCAACATGTG  
1651 TCCTTCCTGA TTCTGTGGTG AAGCCACTGC CTCCATCCAG TGTGAAAGCA  
1701 GAAATTACTA TAAACATTGG ATTATTGAAA ATATCTTGGG AAAAGCCAGT  
20 1751 CTTTCCAGAG AATAACCTTC AATTCCAGAT TCGCTATGGT TTAAGTGGAA  
1801 AAGAAGTACA ATGGAAGATG TATGAGGTTT ATGATGCAA ATCAAAATCT  
1851 GTCAGTCTCC CAGTTCCAGA CTTGTGTGCA GTCTATGCTG TTCAGGTGCG  
25 1901 CTGTAAGAGG CTAGATGGAC TGGGATATTG GAGTAATTGG AGCAATCCAG  
1951 CCTACACAGT TGTCAATGGAT ATAAAAGTTC CTATGAGAGG ACCTGAATTT  
30 2001 TGGAGAATAA TTAATGGAGA TACTATGAAA AAGGAGAAAA ATGTCACTTT  
2051 ACTTTGGAAG CCCCTGATGA AAAATGACTC ATTGTGCAGT GTTCAGAGAT  
2101 ATGTGATAAA CCATCATACT TCCTGCAATG GAACATGGTC AGAAGATGTG  
35 2151 GGAAATCACA CGAAATTACAC TTTCTGTGG ACAGAGCAAG CACATACTGT  
2201 TACGGTTCTG GCCATCAATT CAATTGGTGC TTCTGTTGCA AATTTAATT  
40 2251 TAACCTTTTC ATGGCCTATG AGCAAAGTAA ATATCGTGCA GTCACTCAGT  
2301 GCTTATCCTT TAAACAGCAG TTGTGTGATT GTTCCTGGA TACTATCACC  
2351 CAGTGATTAC AAGCTAATGT ATTTTATTAT TGAGTGGAAA AATCTTAATG  
45 2401 AAGATGGTGA AATAAAATGG CTTAGAATCT CTTCATCTGT TAAGAAGTAT  
2451 TATATCCATG ATCATTAT CCCCCATTGAG AAGTACCAAGT TCAGTCTTTA  
50 2501 CCCAATATTT ATGGAAGGAG TGGGAAAACC AAAGATAATT AATAGTTCA  
2551 CTCAAGATGA TATTGAAAAA CACCAGAGTG ATGCAGGTTT ATATGTAATT

2601 GTGCCAGTAA TTATTCCTC TTCCATCTTA TTGCTTGAA CATTATTAAT  
2651 ATCACACCAA AGAATGAAAA AGCTATTTG GGAAGATGTT CCGAACCCCA  
5 2701 AGAATTGTTCTGGGCACAA GGACTTAATT TTCAGAAGAG AACGGACATT  
2751 CTTTGAAGTC TAATCATGAT CACTACAGAT GAACCCAATG TGCCAACTTC  
2801 CCAACAGTCT ATAGAGTATT AGAAGATT TACATTTGA AGAAGGGGAG  
10 2851 CAAATCTAAA AAAAATTCAG TTGAACCTCT GAGAGTTAAC ATATGGTGGAA  
2901 TTATGTTGAT TTAGAACTTA AAATAGATGT GTAAATTTGG GTTCAAAATG  
15 2951 TAGATTTGAG TCCAGTTGG ATGTGTGATT AATTTCAAA TCATCTAAAG  
3001 TTTAAAAGTA GTATTCATGA TTTCTGGCTT TTGATTTGCC ATATTCTGG  
3051 TCATAAAACA TTAAGAAAAT TATGGCTGTT GCTGTCATTA CATATCTATT  
20 3101 AAATGTCATC AAATATGTAG TAGACAATT TGTAATTAGG TGAACCTCAA  
3151 AACTGCAACA TCTGACAAAT TGCTTTAAAA ATACAATGAT TAT

Human OB Receptor "B" Amino Acid Sequence (Seq. ID No. 3 (Amino Acid)):

5    1    MICQKFCVVL LHWEFIYVIT AFNLSYPITP WRFKLSCMPP NSTYDYFLLP  
      51    AGLSKNTSNS NGHYETAVEP KFNSSGTHFS NLSKTTFHCC FRSEQDRNCS  
10    101    LCADNIEGKT FVSTVNSLVF QQIDANWNIQ CWLKGDLKLF ICYVESLFKN  
      151    LFRNYNYKVH LLYVLPEVLE DSPLVPQKGS FQMVHCNCBV HECCECLVPV  
20    201    PTAKLNDTLL MCLKITSGGV IFQSPLMSVQ PINMVKPDPPI LGLHMEITDD  
      251    GNLKISWSSP PLVPFPLQYQ VKYSENSTTV IREADKIVSA TSLLVDSILP  
      301    GSSYEVQVRG KRLDGPGIWS DWSTPRVFTT QDVIYFPPKI LTSVGGSNVSE  
      351    HCIYKKENKI VPSKEIVWWM NLAEKIPQSQ YDVVSDHVSX VTFFNLNETK  
25    401    PRGKFTYDAV YCCNEHECHH RYAELYVIDV NINISCETDG YLTCKMTCRWS  
      451    TSTIQSLAES TLQLRYHRSS LYCSDIPSIIH PISEPKDCYL QSDGFYECIF  
25    501    QPIFLLSGYT MWIRINHSLG SLDSPPTCVL PDSVVVKPLPP SSVKAEITIN  
      551    IGLLKISWEK PVFPENNLLQF QIRYGLSGKE VQWKMYEVYD AKSKSVSLPV  
30    601    PDLCAVYAVQ VRCKRLDGLG YWSNWSNPAY TVVMDIKVPM RGPEFWRIIN  
      651    GDTMKKEKNV TLLWKPLMKN DSLCSVQRYY INHHHTSCNGT WSEDVGNHTK  
70    701    FTFLWTEQAH TVTVLAINSI GASVANFNLT FSWPMSKVNI VQSL SAYPLN  
35    751    SSCVIVSWIL SPSDYKLMYF IIEWKNLNED GEIKWLRSS SVKKYYIHHDH  
      801    FIPIEKYQFS LYPIFMEGVG KPKIINSFTQ DDIEKHQSDA GLYVIVPVII  
40    851    SSSILLLGTL LISHQRMKKL FWEDVPNPKN CSWAQGLNFQ KKRLSIFLSS  
      901    IQHQ\*HVVLF FWSLKQFQKI SVLIHHGKIK MR\*CQQLWSL YFQQQILKRV  
      951    LFVLVTSSTV LTSLRLRVLR \*PMRTKARDN PLLNTPR\*SA TLNQVKLVK

Human OB Receptor "B" DNA Sequence (Seq. ID No. 4 (DNA)):

1 CCGCCGCCAT CTCTGCCTTC GGTCGAGTTG GACCCCCGGA TCAAGGTGTA  
5 51 CTTCTCTGAA GTAAGATGAT TTGTCAAAAA TTCTGTGTGG TTTTGTACA  
10 101 TTGGGAATT ATTATATGTGA TAACTGCGTT TAACTTGTCA TATCCAATTA  
15 151 CTCCTTGGAG ATTTAACGTTG TCTTGCATGC CACCAAATT AACCTATGAC  
20 201 TACTTCCTT TGCCCTGCTGG ACTCTCAAAG AATACTCAA ATTCAATGG  
25 251 ACATTATGAG ACAGCTGTTG AACCTAAGT TAATTCAAGT GGTACTCACT  
30 301 TTTCTAACCT ATCCAAAACA ACTTTCCACT GTTGCTTCG GAGTGAGCAA  
35 351 GATAGAAACT GCTCCTTATG TGCAAGACAAC ATTGAAGGAA AGACATTTGT  
40 401 TTCAACAGTA AATTCTTAG TTTTCAACA AATAGATGCA AACTGGAACA  
45 451 TACAGTGCTG GCTAAAAGGA GACTTAAAT TATTCAATCTG TTATGTGGAG  
50 501 TCATTATTTA AGAATCTATT CAGGAATTAT AACTATAAGG TCCATCTTTT  
55 551 ATATGTTCTG CCTGAAGTGT TAGAAGATTAC ACCTCTGGTT CCCCAAAAAG  
60 601 GCAGTTTCAC GATGGTTCAC TGCAATTGCA GTGTCATGA ATGTTGTGAA  
65 651 TGTCTTGTGC CTGTGCCAAC AGCCAAACTC AACGACACTC TCCTTATGTG  
70 701 TTTGAAAATC ACATCTGGTG GAGTAATTT CCAGTCACCT CTAATGTCAG  
75 751 TTCAGCCCCAT AAATATGGTG AAGCCTGATC CACCATTAGG TTTGCATATG  
80 801 GAAATCACAG ATGATGGTAA TTTAAAGATT TCTTGGTCCA GCCCACCATT  
85 851 GGTACCATT CCACTTCAAT ATCAAGTGAA ATATTCAAGG AATTCTACAA  
90 901 CAGTTATCAG AGAAGCTGAC AAGATTGTCT CAGCTACATC CCTGCTAGTA  
95 951 GACAGTATAC TTCCCTGGGTC TTCGTATGAG GTTCAGGTGA GGGGCAAGAG  
100 1001 ACTGGATGGC CCAGGAATCT GGAGTGACTG GAGTACTCCT CGTGTCTTTA  
105 1051 CCACACAAAGA TGTCAATATAC TTTCCACCTA AAATTCTGAC AAGTGTGGG  
110 1101 TCTAATGTTT CTTTCACTG CATCTATAAG AAGGAAAACA AGATTGTTCC  
115 1151 CTCAAAAGAG ATTGTTGGT GGATGAATT AGCTGAGAAA ATTCCCTAAA  
120 1201 GCCAGTATGA TGTTGTGAGT GATCATGTTA GCAAAGTTAC TTTTTCAAT  
125 1251 CTGAATGAAA CCAAACCTCG AGGAAAGTTT ACCTATGATG CAGTGTACTG

1301 CTGCAATGAA CATGAATGCC ATCATCGCTA TGCTGAATTA TATGTGATTG  
1351 ATGTCAATAT CAATATCTCA TGTGAAACTG ATGGGTACTT AACTAAAATG  
5 1401 ACTTGCAGAT GGTCAACCAG TACAATCCAG TCACCTGCGG AAAGCACTTT  
1451 GCAATTGAGG TATCATAGGA GCAGCCTTA CTGTTCTGAT ATTCCATCTA  
10 1501 TTCATCCCAT ATCTGAGCCC AAAGATTGCT ATTTGCAGAG TGATGGTTT  
1551 TATGAATGCA TTTTCCAGCC AATCTTCCTA TTATCTGGCT ACACAATGTG  
1601 GATTAGGATC AATCACTCTC TAGGTTCACT TGACTCTCCA CCAACATGTG  
15 1651 TCCTTCCTGA TTCTGTGGTG AAGCCACTGC CTCCATCCAG TGTGAAAGCA  
1701 GAAATTACTA TAAACATTGG ATTATTGAAA ATATCTGGG AAAAGCCAGT  
20 1751 CTTTCCAGAG AATAACCTTC AATTCCAGAT TCGCTATGGT TTAAGTGGAA  
1801 AAGAAGTACA ATGGAAGATG TATGAGGTTT ATGATGCAAATC  
1851 GTCAGTCTCC CAGTTCCAGA CTTGTGTGCA GTCTATGCTG TTCAGGTGCG  
25 1901 CTGTAAGAGG CTAGATGGAC TGGGATATTG GAGTAATTGG AGCAATCCAG  
1951 CCTACACAGT TGTCAATGGAT ATAAAAGTTC CTATGAGAGG ACCTGAATTT  
30 2001 TGGAGAATAA TTAATGGAGA TACTATGAAA AAGGAGAAAA ATGTCACCTT  
2051 ACTTTGGAAG CCCCTGATGA AAAATGACTC ATTGTGCAGT GTTCAGAGAT  
2101 ATGTGATAAA CCATCATACT TCCTGCAATG GAACATGGTC AGAAGATGTG  
35 2151 GGAAATCACA CGAAATTACAC TTTCTGTGG ACAGAGCAAG CACATACTGT  
2201 TACGGTTCTG GCCATCAATT CAATTGGTGC TTCTGTTGCA AATTTAATT  
40 2251 TAACCTTTTC ATGGCCTATG AGCAAAGTAA ATATCGTGCA GTCACTCAGT  
2301 GCTTATCCTT TAAACAGCAG TTGTGTGATT GTTCCTGGA TACTATCACC  
2351 CAGTGATTAC AAGCTAATGT ATTTTATTAT TGAGTGGAAA AATCTTAATG  
45 2401 AAGATGGTGA AATAAAATGG CTTAGAATCT CTTCATCTGT TAAGAAGTAT  
2451 TATATCCATG ATCATTAT CCCCCATTGAG AAGTACCAAGT TCAGTCTTTA  
50 2501 CCCAATATTT ATGGAAGGAG TGGGAAAACC AAAGATAATT AATAGTTCA  
2551 CTCAAGATGA TATTGAAAAA CACCAGAGTG ATGCAGGTTT ATATGTAATT

2601 GTGCCAGTAA TTATTCCTC TTCCATCTTA TTGCTTGAA CATTATTAAT  
2651 ATCACACCAA AGAACAAAAA AGCTATTTG GGAAGATGTT CCGAACCCCA  
5 2701 AGAATTGTTCTGGGCACAA GGACTTAATT TTCAGAAGAA ACGTTGAGC  
2751 ATCTTTTAT CAAGCATACA GCATCAGTGA CATGTGGTCC TCTTCTTTG  
2801 GAGCCTGAAA CAATTCAGA AGATATCAGT GTTGATACAT CATGGAAAAA  
10 2851 TAAAGATGAG ATGATGCCAA CAACTGTGGT CTCTCTACTT TCAACAAACAG  
2901 ATCTTGAAA GGGTTCTGTT TGTTTAGTG ACCAGTTCAA CAGTGTAAAC  
15 2951 TTCTCTGAGG CTGAGGGTAC TGAGGTAACC TATGAGGACG AAAGCCAGAG  
3001 ACAACCCTTT GTTAAATACG CCACGCTGAT CAGCAACTCT AAACCAAGTG  
3051 AAACTGGTGA AGA

Human OB Receptor "C" Amino Acid Sequence (Seq. ID No. 5 (Amino Acid)):

5    1    MICQKFCVVL LHWEFIYVIT AFNLSYPITP WRFKLSCMPP NSTYDYFLLP  
     51    AGLSKNTSNS NGHYETAVEP KFNSSGTHFS NLSKTTFHCC FRSEQDRNCS  
    101    LCADNIEGKT FVSTVNSLVF QQIDANWNIQ CWLKGDLKLF ICYVESLFKN  
    151    LFRNYNYKVH LLYVLPEVLE DSPLVPQKGS FQMVHCNCBV HECCECLVPV  
    201    PTAKLNDTLL MCLKITSGGV IFQSPLMSVQ PINMVKPDPPI LGLHMEITDD  
    251    GNLKISWSSP PLVPFPLQYQ VKYSENSTTV IREADKIVSA TSLLVDSILP  
    301    GSSYEVQVRG KRLDGPGIWS DWSTPRVFTT QDVIYFPPKI LTSVGSNVSF  
    351    HCIYKKENKI VPSKEIVWWM NLAEKIPQSQ YDVVSDHVSX VTFFNLNETK  
    401    PRGKFTYDAV YCCNEHECHH RYAELYVIDV NINISCETDG YLTKMTCRWS  
    451    TSTIQSLAES TLQLRYHRSS LYCSDIPSIIH PISEPKDCYL QSDGFYECIF  
    501    QPIFLLSGYT MWIRINHSLG SLDSPPTCVL PDSVVKPLPP SSVKAEITIN  
    551    IGLLKISWEK PVFPENNLOF QIRYGLSGKE VQWKMYEVYD AKSKSVSLPV  
    601    PDLCAVYAVQ VRCKRLDGLG YWSNWSNPAY TVVMDIKVPM RGPEFWRIIN  
    651    GDTMKKEKNV TLLWKPLMKN DSLCSVQRYV INHHTSCNGT WSEDVGNHTK  
    701    FTFLWTEQAH TVTVLAINSI GASVANFNLT FSWPMSKVNI VQSL SAYPLN  
    751    SSCVIVSWIL SPSDYKLMYF IIEWKNLNED GEIKWLRSS SVKKYYIHDH  
    801    FIPIEKYQFS LYPIFMEGVG KPKIINSFTQ DDIEKHQSDA GLYVIVPVII  
    851    SSSILLLGTL LISHQRMKKL FWEDVPNPKN CSWAQGLNFQ KMLEGSMFVK  
    901    SHHHSLISST QGHKHCGRPQ GPLHRKTRDL CSLVYLLTLP PLLSYDPAKS  
    951    PSVRNTQE\*S IKKKKKKLEG

Human OB Receptor "C" DNA Sequence (Seq. ID No. 6 (DNA)):

1 CCGCCGCCAT CTCTGCCTTC GGTCGAGTTG GACCCCCGGA TCAAGGTGTA  
5 51 CTTCTCTGAA GTAAGATGAT TTGTAAAAAA TTCTGTGTGG TTTTGTACA  
10 101 TTGGGAATT ATTATGTGA TAACTGCGTT TAACTTGTCA TATCCAATT  
15 151 CTCCTGGAG ATTTAAGTTG TCTTGCATGC CACCAAATT AACCTATGAC  
20 201 TACTTCCTT TGCGCTGCTGG ACTCTCAAAG AATACTCAA ATTGAAATGG  
25 251 ACATTATGAG ACAGCTGTTG AACCTAAGTT TAATTCAAGT GGTACTCACT  
30 301 TTTCTAACTT ATCCAAAACA ACTTTCCACT GTTGCTTCG GAGTGAGCAA  
35 351 GATAGAAACT GCTCCTTATG TGCGACAAAC ATTGAAGGAA AGACATTGT  
40 401 TTCAACAGTA AATTCTTAG TTTTCAACA AATAGATGCA AACTGGAACA  
45 451 TACAGTGCTG GCTAAAAGGA GACTTAAAAT TATTCACTG TTATGTGGAG  
50 501 TCATTATTTA AGAATCTATT CAGGAATTAT AACTATAAGG TCCATCTTT  
55 551 ATATGTTCTG CCTGAAGTGT TAGAAGATTAC ACCTCTGGTT CCCCAAAAAG  
60 601 GCAGTTTCA GATGGTTCAC TGCAATTGCA GTGTTCATGA ATGTTGTGAA  
65 651 TGTCTTGTGC CTGTGCCAAC AGCCAAACTC AACGACACTC TCCTTATGTG  
70 701 TTTGAAAATC ACATCTGGTG GAGTAATTTC CCAGTCACCT CTAATGTCAG  
75 751 TTCAGCCCAT AAATATGGTG AAGCCTGATC CACCATTAGG TTTGCATATG  
80 801 GAAATCACAG ATGATGGTAA TTTAAAGATT TCTTGGTCCA GCCCACCATT  
85 851 GGTACCATT CCACTTCAAT ATCAAGTGAA ATATTCAAGG AATTCTACAA  
90 901 CAGTTATCAG AGAAGCTGAC AAGATTGTCT CAGCTACATC CCTGCTAGTA  
95 951 GACAGTATAC TTCCCTGGGTC TTCGTATGAG GTTCAGGTGA GGGGCAAGAG  
100 1001 ACTGGATGGC CCAGGAATCT GGAGTGACTG GAGTACTCCT CGTGTCTTTA  
105 1051 CCACACAAAGA TGTCAATATAC TTTCCACCTA AAATTCTGAC AAGTGTGGG  
110 1101 TCTAATGTTT CTTTCACTG CATCTATAAG AAGGAAAACA AGATTGTTCC  
115 1151 CTCAAAAGAG ATTGTTGGT GGATGAATT AGCTGAGAAA ATTCCCTCAA  
120 1201 GCCAGTATGA TGTTGTGAGT GATCATGTTA GCAAAGTTAC TTTTTCAAT

1251 CTGAATGAAA CCAAACCTCG AGGAAAGTTT ACCTATGATG CAGTGTACTG  
1301 CTGCAATGAA CATGAATGCC ATCATCGCTA TGCTGAATTA TATGTGATTG  
5 1351 ATGTCAATAT CAATATCTCA TGTGAAACTG ATGGGTACTT AACTAAAATG  
1401 ACTTGCAGAT GGTCAACCAG TACAATCCAG TCACTTGCGG AAAGCACTTT  
1451 GCAATTGAGG TATCATAGGA GCAGCCTTA CTGTTCTGAT ATTCCATCTA  
10 1501 TTCATCCCAT ATCTGAGCCC AAAGATTGCT ATTTGCAGAG TGATGGTTT  
1551 TATGAATGCA TTTTCAGGCC AATCTCCTA TTATCTGGCT ACACAATGTG  
15 1601 GATTAGGATC AATCACTCTC TAGGTTCACT TGACTCTCCA CCAACATGTG  
1651 TCCTTCCTGA TTCTGTGGTG AAGCCACTGC CTCCATCCAG TGTGAAAGCA  
1701 GAAATTACTA TAAACATTGG ATTATTGAAA ATATCTTGGG AAAAGCCAGT  
20 1751 CTTTCCAGAG AATAACCTTC AATTCCAGAT TCGCTATGGT TTAAGTGGAA  
1801 AAGAAGTACA ATGGAAGATG TATGAGGTTT ATGATGCAA ATCAAAATCT  
25 1851 GTCAGTCTCC CAGTTCCAGA CTTGTGTGCA GTCTATGCTG TTCAGGTGCG  
1901 CTGTAAGAGG CTAGATGGAC TGGGATATTG GAGTAATTGG AGCAATCCAG  
1951 CCTACACAGT TGTATGGAT ATAAAAGTTC CTATGAGAGG ACCTGAATTT  
30 2001 TGGAGAATAA TTAATGGAGA TACTATGAAA AAGGAGAAAA ATGTCACHTT  
2051 ACTTTGGAAG CCCCTGATGA AAAATGACTC ATTGTGCAGT GTTCAGAGAT  
35 2101 ATGTGATAAA CCATCATACT TCCTGCAATG GAACATGGTC AGAAGATGTG  
2151 GGAAATCACA CGAAATTACAC TTTCTGTGG ACAGAGCAAG CACATACTGT  
2201 TACGGTTCTG GCCATCAATT CAATTGGTG TTCTGTTGCA AATTTAATT  
40 2251 TAACCTTTTC ATGGCCTATG AGCAAAGTAA ATATCGTGCA GTCACTCAGT  
2301 GCTTATCCTT TAAACAGCAG TTGTGTGATT GTTCCTGGA TACTATCACC  
45 2351 CAGTGATTAC AAGCTAATGT ATTTTATTAT TGAGTGGAAA AATCTTAATG  
2401 AAGATGGTGA AATAAAATGG CTTAGAATCT CTTCATCTGT TAAGAAGTAT  
2451 TATATCCATG ATCATTAT CCCCATTGAG AAGTACCAAGT TCAGTCTTTA  
50 2501 CCCAATATTT ATGGAAGGAG TGGGAAAACC AAAGATAATT AATAGTTCA  
2551 CTCAGATGA TATTGAAAAA CACCAGAGTG ATGCAGGTTT ATATGTAATT

2601 GTGCCAGTAA TTATTCCTC TTCCATCTTA TTGCTTGGAA CATTATTAAT  
2651 ATCACACCAA AGAACGAAAA AGCTATTTG GGAAGATGTT CCGAACCCCA  
5 2701 AGAATTGTTC CTGGGCACAA GGACTTAATT TTCAGAAGAT GCTTGAAGGC  
2751 AGCATGTTCG TTAAGAGTCA TCACCACTCC CTAATCTCAA GTACCCAGGG  
10 2801 ACACAAACAC TGCGGAAGGC CACAGGGTCC TCTGCATAGG AAAACCAGAG  
2851 ACCTTTGTTC ACTTGTTTAT CTGCTGACCC TCCCTCCACT ATTGTCTTAT  
2901 GACCCCTGCCA AATCCCCCTC TGTGAGAAC ACCCAAGAAT GATCAATAAA  
15 2951 AAAAAAAAAA AAAAAACTCG AGGGGG

Human OB Receptor "D" Amino Acid Sequence (Sequence ID No. 7)

1 MICQKFCVVL LHWEFIYVIT AFNLSYPITP WRFKLSCMPP NSTYDYFLLP  
5 AGLSKNTSNS NGHYETAVEP KFNSSGTHFS NLSKTTFHCC FRSEQDRNCS  
10 101 LCADNIEGKT FVSTVNSLVF QQIDANWNIQ CWLKGDLKLF ICYVESLFKN  
15 151 LFRNYNYKVH LLYVLPEVLE DSPLVPQKGS FQMVHCNCBV HECCECLVPV  
20 201 PTAKLNDTLL MCLKITSGGV IFQSPLMSVQ PINMVKPDPPI LGLHMEITDD  
25 251 GNLKISWSSP PLVPFPLQYQ VKYSENSTTV IREADKIVSA TSLLVDSILP  
30 301 GSSYEVQVRG KRLDGPGIWS DWSTPRVFTT QDVIIYFPPKI LTSVGSNVSE  
35 351 HCIYKKENKI VPSKEIVWWM NLAEKIPQSQ YDVVSDHVSX VTFFNLNETK  
40 401 PRGKFTYDAV YCCNEHECHH RYAELYVIDV NINISCETDG YLTCKMTCRWS  
45 451 TSTIQSLAES TLQLRYHRSS LYCSDIPSIIH PISEPKDCYL QSDGFYECIF  
50 501 QPIFLLSGYT MWIRINHSLG SLDSPPTCVL PDSVVKPLPP SSVKAEITIN  
55 551 IGLLKISWEK PVFPENNLLQF QIRYGLSGKE VQWKMYEVYD AKSKSVSLPV  
60 601 PDLCAVYAVQ VRCKRLDGLG YWSNWSNPAY TVVMDIKVPM RGPEFWRIIN  
65 30 651 GDTMKKEKNV TLLWKPLMKN DSLCSVQRYY INHHTSCNGT WSEDVGNHTK  
70 701 FTFLWTEQAH TVTVLAINS GASVANFNLT FSWPMSKVNI VQSL SAYPLN  
75 751 SSCVIVSWIL SPSDYKLMYF IIIEWKNLNED GEIKWLRSS SVKKYYIHHDH  
80 35 801 FIPIEKYQFS LYPIFMEVGK KPKIINSFTQ DDIEKHQSDA GLYVIVPVII  
85 851 SSSILLGTL LISHORMKKL FWEDVPNPKN CSWAQGLNFQ KPETFEHLFI  
90 40 901 KHTASVTCPGP LLLEPETISE DISVDTSWKN KDEMMPTTVV SLLSTTDLEK  
95 951 GSVCISDQFN SVNFSSEAEGT EVTYEDESQR QPFVKYATLI SNSKPSETGE  
100 1001 EQGLINSSVT KCFSSKNSPL KDSFSNSSWE IEAQAFFILS DQHPNIISPH  
105 45 1051 LTFSEGLDEL LKLEGNFPEE NNDKKSIYYL GTTSIKKRES GVLLTDKSRV  
110 1101 SCPFPAPCLF TDIRVLQDSC SHFVENNINL GTSSKKTFAS YMPQFQTCST  
115 50 1151 QTHKIMENKM CDLTV\*FH\*R NLQICVIMGN IKCNRL\*LWV GERKETRVKF  
120 1201 ENNCSK\*KKK KKNSRPARPD

Human OB Receptor "D" Nucleic Acid Sequence (Sequence ID No. 8)

1 GCGGCCGCCA GTGTGATGGA TATCTGCAGA ATTGGCTTT CTCTGCCCTC  
5 51 GGTGAGTTG GACCCCCGGA TCAAGGTGTA CTTCTCTGAA GTAAGATGAT  
10 101 TTGTCAAAAA TTCTGTGTGG TTTTGTACA TTGGGAATT ATTATGTGA  
15 151 TAACTGCGTT TAACTTGTCA TATCCAATT A CTCCCTGGAG ATTTAAGTTG  
20 201 TCTTGCATGC CACCAAATT AACCTATGAC TACTCCTTT TGCCTGCTGG  
25 251 GCTCTCAAAG AATACTCAA ATTGAATGG ACATTATGAG ACAGCTGTTG  
30 301 AACCTAAGTT TAATTCAAGT GGTACTCACT TTTCTAACCT ATCCAAAACA  
35 351 ACTTTCCACT GTTGCTTCG GAGTGAGCAA GATAGAAACT GCTCCTTATG  
40 401 TGCAGACAAAC ATTGAAGGAA AGACATTGT TTCAACAGTA AATTCTTAG  
45 451 TTTTCAACA AATAGATGCA AACTGGAACA TACAGTGCTG GCTAAAAGGA  
50 501 GACTTAAAAT TATTCACTG TTATGTGGAG TCATTATTTA AGAATCTATT  
55 551 CAGGAATTAT AACTATAAGG TCCATCTTT ATATGTTCTG CCTGAAGTGT  
60 601 TAGAAGATTC ACCTCTGGTT CCCCAAAAAG GCAGTTTCA GATGGTTCAC  
65 651 TGCAATTGCA GTGTCACGA ATGTTGTGAA TGTCTTGTGC CTGTGCCAAC  
70 701 AGCCAAACTC AACGACACTC TCCTTATGTG TTTGAAAATC ACATCTGGTG  
75 751 GAGTAATTTC CCAGTCACCT CTAATGTCAG TTCAGCCCATT AAATATGGTG  
80 801 AAGCCTGATC CACCATTAGG TTTGCATATG GAAATCACAG ATGATGGTAA  
85 851 TTTAAAGATT TCTTGGTCCA GCCCACCATT GGTACCATT CCACCTCAAT  
90 901 ATCAAGTGAA ATATTCAAGAG AATTCTACAA CAGTTATCAG AGAAGCTGAC  
95 951 AAGATTGTCT CAGCTACATC CCTGCTAGTA GACAGTATAAC TTCCCTGGTCA  
100 1001 TTCGTATGAG GTTCAGGTGA GGGGCAAGAG ACTGGATGGC CCAGGAATCT  
105 1051 GGAGTGACTG GAGTACTCCT CGTGTCTTTA CCACACAAGA TGTCAATATAC  
110 1101 TTTCCACCTA AAATTCTGAC AAGTGTGGG TCTAATGTTT CTTTCACTG  
115 1151 CATCTATAAG AAGGAAAACA AGATTGTTCC CTCAAAAGAG ATTGTTGGT  
120 1201 GGATGAATT AGCTGAGAAA ATTCCCTAAA GCCAGTATGA TGTTGTGAGT  
125 1251 GATCATGTTA GCAAAGTTAC TTTTTCAAT CTGAATGAAA CCAAACCTCG

1301 AGGAAAGTTT ACCTATGATG CAGTGTACTG CTGCAATGAA CATGAATGCC  
1351 ATCATCGCTA TGCTGAATTA TATGTGATTG ATGTCAATAT CAATATCTCA  
5 1401 TGTGAAACTG ATGGGTACTT AACTAAAATG ACTTGCAGAT GGTCAACCAG  
1451 TACAATCCAG TCACTTGCAG AAAGCACTTT GCAATTGAGG TATCATAGGA  
10 1501 GCAGCCTTA CTGTTCTGAT ATTCCATCTA TTCATCCCCT ATCTGAGCCC  
1551 AAAGATTGCT ATTTGCAGAG TGATGGTTT TATGAATGCA TTTTCCAGCC  
1601 AATCTTCCTA TTATCTGGCT ACACAATGTG GATTAGGATC AATCACTCTC  
15 1651 TAGGTTCACT TGACTCTCCA CCAACATGTG TCCTTCCTGA TTCTGTGGTG  
1701 AAGCCACTGC CTCCATCCAG TGTGAAAGCA GAAATTACTA TAAACATTGG  
20 1751 ATTATTGAAA ATATCTTGGG AAAAGCCAGT CTTTCCAGAG AATAACCTTC  
1801 AATTCCAGAT TCGCTATGGT TTAAGTGGAA AAGAAGTACA ATGGAAGATG  
1851 TATGAGGTTT ATGATGCAA ATCAAAATCT GTCAGTCTCC CAGTCCAGA  
25 1901 CTTGTGTGCA GTCTATGCTG TTCAGGTGCG CTGTAAGAGG CTAGATGGAC  
1951 TGGGATATTG GAGTAATTGG AGCAATCCAG CCTACACAGT TGTCATGGAT  
30 2001 ATAAAAGTTC CTATGAGAGG ACCTGAATT TGGAGAATAA TTAATGGAGA  
2051 TACTATGAAA AAGGAGAAAA ATGTCACTTT ACTTTGGAAG CCCCTGATGA  
2101 AAAATGACTC ATTGTGCAGT GTTCAGAGAT ATGTGATAAA CCATCATACT  
35 2151 TCCTGCAATG GAACATGGTC AGAAGATGTG GGAAATCACA CGAAATTAC  
2201 TTTCCCTGTGG ACAGAGCAAG CACATACTGT TACGGTTCTG GCCATCAATT  
40 2251 CAATTGGTGC TTCTGTTGCA AATTTAATT TAACCTTTTC ATGGCCTATG  
2301 AGCAAAGTAA ATATCGTGCA GTCACTCAGT GCTTATCCTT TAAACAGCAG  
2351 TTGTGTGATT GTTCCTGGA TACTATCACC CAGTGATTAC AAGCTAATGT  
45 2401 ATTTTATTAT TGAGTGGAAA AATCTTAATG AAGATGGTGA AATAAAATGG  
2451 CTTAGAATCT CTTCATCTGT TAAGAAGTAT TATATCCATG ATCATTAT  
50 2501 CCCCCATTGAG AAGTACCACT TCAGTCTTA CCCAATATT ATGGAAGGAG  
2551 TGGGAAAACC AAAGATAATT AATAGTTCA CTCAAGATGA TATTGAAAAA

2601 CACCAAGAGTG ATGCAGGTTT ATATGTAATT GTGCCAGTAA TTATTCCTC  
2651 TTCCATCTTA TTGCTTGGAA CATTATTAAT ATCACACCAA AGAATGAAAA  
5 2701 AGCTATTTG GGAAGATGTT CGAACCCCCA AGAATTGTTC CTGGGCACAA  
2751 GGACTTAATT TTCAGAAGCC AGAACGTTT GAGCATCTT TTATCAAGCA  
2801 TACAGCATCA GTGACATGTG GTCCTCTTCT TTTGGAGCCT GAAACAATTT  
10 2851 CAGAAGATAT CAGTGTGAT ACATCATGGA AAAATAAAGA TGAGATGATG  
2901 CCAACAACTG TGGTCTCTCT ACTTTCAACA ACAGATCTT GAAAGGGTTC  
15 2951 TGTTGTATT AGTGACCAGT TCAACAGTGT TAACTTCTCT GAGGCTGAGG  
3001 GTACTGAGGT AACCTATGAG GACGAAAGCC AGAGACAACC CTTTGTAAA  
20 3051 TACGCCACGC TGATCAGCAA CTCTAAACCA AGTGAAACTG GTGAAGAAC  
3101 AGGGCTTATA AATAGTCAG TCACCAAGTG CTTCTCTAGC AAAAATTCTC  
3151 CGTTGAAGGA TTCTTCTCT AATAGCTCAT GGGAGATAGA GGCCCAGGCA  
25 3201 TTTTTATAT TATCGGATCA GCATCCCAAC ATAATTCAC CACACCTCAC  
3251 ATTCTCAGAA GGATTGGATG AACTTTGAA ATTGGAGGGA AATTCCTG  
3301 AAGAAAATAA TGATAAAAAG TCTATCTATT ATTTAGGGGT CACCTCAATC  
30 3351 AAAAAGAGAG AGAGTGGTGT GCTTTGACT GACAAGTCAA GGGTATCGTG  
3401 CCCATTCCA GCCCCCTGTT TATTACCGA CATCAGAGTT CTCCAGGACA  
35 3451 GTTGCTCACA CTTGTAGAA AATAATATCA ACTTAGGAAC TTCTAGTAAG  
3501 AAGACTTTG CATCTTACAT GCCTCAATT CAAACTTGTG CTACTCAGAC  
3551 TCATAAGATC ATGGAAAACA AGATGTGTGA CCTAACTGTG TAATCTAGA

Human OB Receptor Protein "D" Chromosomal DNA (Seq. ID No. 9)

|    |             |                   |           |                              |                  |
|----|-------------|-------------------|-----------|------------------------------|------------------|
| 5  |             |                   | Intron 1  | .....taccccccag              | GTG TAC TTC      |
| 10 | CAT TGG G   | gtaagttatgg....   | Intron 2  | .....atatccaaacag AA TTT ATT | Phe Ile<br>15 16 |
|    | His Trp Glu |                   |           |                              |                  |
|    | 12 13 14    |                   |           |                              |                  |
| 15 | CAA ATA G   | gtaagcattago....  | Intron 3  | .....ttttaattcag             | AT GCA AAC       |
|    | Gln Ile Asp |                   |           |                              |                  |
|    | 122 123 124 |                   |           |                              |                  |
| 20 | TAT GTT CT  | gtaagtacccaaa.... | Intron 4  | .....tttcaatatacg            | G CCT GAA        |
|    | Tyr Val Leu |                   |           |                              |                  |
|    | 163 164 165 |                   |           |                              |                  |
| 25 | AAT ATG G   | gtaagttatgc....   | Intron 5  | .....ttttcccttaag            | TG AAG CCT       |
|    | Asn Met Val |                   |           |                              |                  |
|    | 233 234 235 |                   |           |                              |                  |
| 30 | ATC AGA GAA | gtaagtatattt....  | Intron 6  | .....aatatttAACAG            | GCT GAC AAG      |
|    | Ile Arg Glu |                   |           |                              |                  |
|    | 281 282 283 |                   |           |                              |                  |
| 35 | ACA CAA G   | gtaggTTATGTA....  | Intron 7  | .....ccctcattacag            | AT GTC ATA       |
|    | Thr Gln Asp |                   |           |                              |                  |
|    | 330 331 332 |                   |           |                              |                  |
| 40 | GTG ATT G   | gtaagaaaaacag.... | Intron 8  | .....tgTTTCAAATAG            | AT GTC AAT       |
|    | Val Ile Asp |                   |           |                              |                  |
|    | 427 428 429 |                   |           |                              |                  |
| 45 | TAT CAT AG  | gtacgtattatt....  | Intron 9  | .....tatTTTTAAAG             | G AGC AGC        |
|    | Tyr His Arg |                   |           |                              |                  |
|    | 466 467 468 |                   |           |                              |                  |
| 50 | TCT GTG G   | gtatgtcaagct....  | Intron 10 | .....aaaaATTTCAG             | TG AAG CCA       |
|    | Ser Val Val |                   |           |                              |                  |
|    | 533 534 535 |                   |           |                              |                  |
| 55 | CAA TGG AAG | gtacccTTTACT....  | Intron 11 | .....cttATTTACAG             | ATG TAT GAG      |
|    | Gln Trp Lys |                   |           |                              |                  |
|    | 582 583 584 |                   |           |                              |                  |
| 60 | ATA AAA G   | gtctgcagagat....  | Intron 12 | .....gtcattttgcag            | TT CCT ATG       |
|    | Ile Lys Val |                   |           |                              |                  |
|    | 636 637 638 |                   |           |                              |                  |

|    |        |     |     |                   |           |                    |        |     |     |
|----|--------|-----|-----|-------------------|-----------|--------------------|--------|-----|-----|
|    | CTT    | TGG | AAG | gtattcccaatt..... | Intron 13 | .....tatTTactacag  | CCC    | CTG | ATG |
| 5  | Leu    | Trp | Lys |                   |           |                    | Pro    | Leu | Met |
|    | 663    | 664 | 665 |                   |           |                    | 666    | 667 | 668 |
|    | AGC    | AAA | G   | gtaagaagaggt..... | Intron 14 | .....tttcccctcag   | TA     | AAT | ATC |
| 10 | Ser    | Lys | Val |                   |           |                    | Asn    | Ile |     |
|    | 736    | 737 | 738 |                   |           |                    | 739    | 740 |     |
|    | ATC    | CAT | G   | gtaagtttacta..... | Intron 15 | .....tttttccttcag  | AT     | CAT | TTT |
| 15 | Ile    | His | Asp |                   |           |                    | His    | Phe |     |
|    | 797    | 798 | 799 |                   |           |                    | 800    | 801 |     |
|    | ACT    | CAA | G   | gtaaaaattata..... | Intron 16 | .....tttcttttcag   | AT     | GAT | ATT |
| 20 | Thr    | Gln | Asp |                   |           |                    | Asp    | Ile |     |
|    | 829    | 830 | 831 |                   |           |                    | 832    | 833 |     |
|    | CAC    | CAA | AG  | gtattgtacttg..... | Intron 17 | .....tatcctttgttag | A      | ATG | AAA |
| 25 | His    | Gln | Arg |                   |           |                    | Met    | Lys |     |
|    | 864    | 865 | 866 |                   |           |                    | 867    | 868 |     |
|    | TTT    | CAG | AAG | gttgcttttca.....  | Intron 18 | .....ttatctaaacag  | Exon A | AGA | ACG |
| 30 | Phe    | Gln | Lys |                   |           |                    | Arg    | Thr | Asp |
|    | 889    | 890 | 891 |                   |           |                    | 892    | 893 | 894 |
|    | Exon A |     |     |                   |           |                    | Exon D |     |     |
| 35 | AAA    | TAT | GAT | gtacatttgtct..... | Intron 18 | .....cttttcttttag  | CCA    | GAA | ACG |
|    |        |     |     |                   |           |                    | Pro    | Glu | Thr |
|    |        |     |     |                   |           |                    | 892    | 893 | 894 |
|    | Exon B |     |     |                   |           |                    | Exon B |     |     |
| 40 |        |     |     |                   |           |                    | AAA    | CGT | TTG |
|    |        |     |     |                   |           |                    | Lys    | Arg | Leu |
|    |        |     |     |                   |           |                    | 892    | 893 | 894 |
|    | Exon D |     |     |                   |           |                    | Exon C |     |     |
| 45 | GAA    | ACC | AGA | gtatccagtgtt..... | Intron 18 | .....cttttaaacag   | ATG    | CTT | GAA |
|    |        |     |     |                   |           |                    | Met    | Leu | Glu |
|    |        |     |     |                   |           |                    | 892    | 893 | 894 |

Human OB Receptor Protein, Recombinant Secreted Receptor amino acid sequence (Seq. ID. No. 10):

1 MICQKFCVVL LHWEFIYVIT AFNLSYPITP WRFKLSCMPP NSTYDYFLLP  
5 51 AGLSKNTSNS NGHYETAVEP KFNSSGTHFS NLSKTTFHCC FRSEQDRNCS  
10 101 LCADNIEGKT FVSTVNSLVE QQIDANWNIQ CWLKGDLKLF ICYVESLFKN  
15 151 LFRNYNYKVH LLYVLPEVLE DSPLVPQKGS FQMVHCNCBV HECCECLVPV  
20 201 PTAKLNDTLL MCLKITSGGV IFQSPLMSVQ PINMVKPDPPI LGLHMEITDD  
25 251 GNLKISWSSP PLVPFPLQYQ VKYSENSTTV IREADKIVSA TSLLVDSILP  
30 301 GSSYEVQVRG KRLDGPGIWS DWSTPRVFTT QDVIYFPPKI LTSVGSNVSF  
35 351 HCIYKKENKI VPSKEIVWWM NLAEKIPQSQ YDVVSDHVK VTFFNLNETK  
40 401 PRGKFTYDAV YCCNEHECHH RYAELYVIDV NINISCETDG YLTCKMTCRWS  
45 451 TSTIQSLAES TLQLRYHRSS LYCSDIPSIIH PISEPKDCYL QSDGFYECIF  
50 501 QPIFLLSGYT MWIRINHSLG SLDSPPCTVL PDSVVKPLPP SSVKAEITIN  
55 551 IGLLKISWEK PVFPENNLLQF QIRYGLSGKE VQWKMYEVYD AKSKSVSLPV  
60 601 PDLCAVYAVQ VRCKRLDGLG YWSNWSNPAY TVVMDIKVPM RGPEFWRIIN  
65 651 GDTMKKEKNV TLLWKPLMKN DSLCSVQRYY INHHHTSCNGT WSEDVGNHTK  
70 701 FTFLWTEQAH TVTVLAINSI GASVANFNLT FSWPMSKVNI VQSL SAYPLN  
75 751 SSCVIVSWIL SPSDYKLMYF IIEWKNLNED GEIKWLRSS SVKKYYIHDH  
80 801 FIPIEKYQFS LYPIFMEGVG KPKIINSFTQ DDIEKHQSD

Human OB Receptor Protein, Recombinant Secreted Receptor DNA sequence (Seq. ID. No. 11):

5    1    GCGGCCGCCA GTGTGATGGA TATCTGCAGA ATTCCGGCTTT CTCTGCCCTTC  
51    51    GGTCGAGTTG GACCCCCGGA TCAAGGTGTA CTTCTCTGAA GTAAGATGAT  
101   101    TTGTCAAAAA TTCTGTGTGG TTTTGTACA TTGGGAATT ATTATGTGA  
10    151    TAACTGCGTT TAACTTGTCA TATCCAATT A CTCCTTGGAG ATTTAAGTTG  
201   201    TCTTGCATGC CACCAAATTC AACCTATGAC TACTCCTTT TGCCTGCTGG  
15    251    GCTCTCAAAG AATACTCAA ATTCAATGG ACATTATGAG ACAGCTGTTG  
301   301    AACCTAAGTT TAATTCAAGT GGTACTCACT TTTCTAACTT ATCCAAAACÀ  
351   351    ACTTTCCACT GTTGCTTTCG GAGTGAGCAA GATAGAAACT GCTCCTTATG  
20    401    TGCAGACAAAC ATTGAAGGAA AGACATTGT TTCAACAGTA AATTCTTAG  
451   451    TTTTCAACA AATAGATGCA AACTGGAACA TACAGTGCTG GCTAAAAGGA  
25    501    GACTTAAAAT TATTCACTTG TTATGTGGAG TCATTATTTA AGAATCTATT  
551   551    CAGGAATTAT AACTATAAGG TCCATCTTTT ATATGTTCTG CCTGAAGTGT  
601   601    TAGAAGATTC ACCTCTGGTT CCCCAAAAAG GCAGTTTCA GATGGTTCAC  
30    651    TGCAATTGCA GTGTTCACGA ATGTTGTGAA TGTCTTGTGC CTGTGCCAAC  
701   701    AGCCAAACTC AACGACACTC TCCTTATGTG TTTGAAAATC ACATCTGGTG  
35    751    GAGTAATTTC CCAGTCACCT CTAATGTCAG TTCAGCCCCTT AAATATGGTG  
801   801    AAGCCTGATC CACCATTAGG TTTGCATATG GAAATCACAG ATGATGGTAA  
851   851    TTAAAGATT TCTTGGTCCA GCCCACCATT GGTACCAATT CCACCTCAAT  
40    901    ATCAAGTGAA ATATTCAAGAG AATTCTACAA CAGTTATCAG AGAAGCTGAC  
951   951    AAGATTGTCT CAGCTACATC CCTGCTAGTA GACAGTATAC TTCCCTGGGTC  
45    1001    TTCTGTATGAG GTTCAGGTGA GGGGCAAGAG ACTGGATGGC CCAGGAATCT  
1051   1051    GGAGTGACTG GAGTACTCCT CGTGTCTTTA CCACACAAAGA TGTCAATATAC  
1101   1101    TTTCCACCTA AAATTCTGAC AAGTGTGGG TCTAATGTTT CTTCACGTG  
50    1151    CATCTATAAG AAGGAAAACA AGATTGTTCC CTCAAAAGAG ATTGTTGGT  
1201   1201    GGATGAATT AGCTGAGAAA ATTCCCTAAA GCCAGTATGA TGTTGTGAGT

1251 GATCATGTTA GCAAAGTTAC TTTTTCAAT CTGAATGAAA CCAAACCTCG  
1301 AGGAAAGTTT ACCTATGATG CAGTGTACTG CTGCAATGAA CATGAATGCC  
5 1351 ATCATCGCTA TGCTGAATT AATGTGATTG ATGTCAATAT CAATATCTCA  
1401 TGTGAAACTG ATGGGTACTT AACTAAAATG ACTTGCAGAT GGTCAACCAG  
10 1451 TACAATCCAG TCACTTGCGG AAAGCACTTT GCAATTGAGG TATCATAGGA  
1501 GCAGCCTTTA CTGTTCTGAT ATTCCATCTA TTCATCCCAT ATCTGAGCCC  
1551 AAAGATTGCT ATTTGCAGAG TGATGGTTT TATGAATGCA TTTTCCAGCC  
1601 AATCTTCCTA TTATCTGGCT ACACAATGTG GATTAGGATC AATCACTCTC  
1651 TAGGTTCACT TGACTCTCCA CCAACATGTG TCCTTCCTGA TTCTGTGGTG  
20 1701 AAGCCACTGC CTCCATCCAG TGTGAAAGCA GAAATTACTA TAAACATTGG  
1751 ATTATTGAAA ATATCTTGGG AAAAGCCAGT CTTTCCAGAG AATAACCTTC  
1801 AATTCCAGAT TCGCTATGGT TTAAGTGGAA AAGAAGTACA ATGGAAGATG  
25 1851 TATGAGGTTT ATGATGCAAATCCT GTCAGTCTCC CAGTTCCAGA  
1901 CTTGTGTGCA GTCTATGCTG TTCAGGTGCG CTGTAAGAGG CTAGATGGAC  
30 1951 TGGGATATTG GAGTAATTGG AGCAATCCAG CCTACACAGT TGTCAATGGAT  
2001 ATAAAAGTTC CTATGAGAGG ACCTGAATT TGGAGAATAA TTAATGGAGA  
2051 TACTATGAAA AAGGAGAAAA ATGTCACTTT ACTTTGGAAG CCCCTGATGA  
35 2101 AAAATGACTC ATTGTGCAGT GTTCAGAGAT ATGTGATAAA CCATCATACT  
2151 TCCTGCAATG GAACATGGTC AGAAGATGTG GGAAATCACA CGAAATTAC  
40 2201 TTTCCTGTGG ACAGAGCAAG CACATACTGT TACGGTTCTG GCCATCAATT  
2251 CAATTGGTGC TTCTGTTGCA AATTTAATT TAACCTTTTC ATGGCCTATG  
2301 AGCAAAGTAA ATATCGTGCA GTCACTCAGT GCTTATCCTT TAAACAGCAG  
45 2351 TTGTGTGATT GTTCCTGGA TACTATCACC CAGTGATTAC AAGCTAATGT  
2401 ATTTTATTAT TGAGTGGAAA AATCTTAATG AAGATGGTGA AATAAAATGG  
50 2451 CTTAGAATCT CTTCATCTGT TAAGAAGTAT TATATCCATG ATCATTTAT  
2501 CCCCCATTGAG AAGTACCAAGT TCAGTCTTTA CCCAATATTT ATGGAAGGAG

A-382A

- 85 -

2551 TGGGAAAACC AAAGATAATT AATAGTTCA CTCAAGATGA TATTGAAAAA

2601 CACCA<sup>G</sup>AGTG ATTGATAAGG ATCC }

Human OB Receptor Protein, Recombinant Secreted Receptor DNA sequence with C-terminal FLAG (Seq. ID. No. 12):

5           1       CCATTGAAGT CAATGGGAGT TTGTTTGCG ACCAAAATCA ACGGGGATT  
51          51      CCAAAATGTC GTAATAACCC CGCCCCGTTG ACGCAAATGG GCGGTAGGCG  
10         101     TGTACGGTGG GAGGTCTATA TAAGCAGAGC TCGTTTAGTG AACCGTCAGA  
15         151     TCTCTAGAACG CTGGGTACCA GCTGCTAGCA AGCTTGCTAG CGGCCGCCAG  
20         201     TGTGATGGAT ATCTGCAGAA TTCGGCTTTC TCTGCCTTCG GTCGAGTTGG  
15         251     ACCCCCGGAT CAAGGTGTAC TTCTCTGAAG TAAGATGATT TGTCAAAAT  
30         301     TCTGTGTGGT TTTGTTACAT TGGGAATTAA TTTATGTGAT AACTGCGTTT  
20         351     AACTTGTCAT ATCCAATTAC TCCTTGGAGA TTTAAGTTGT CTTGCATGCC  
40         401     ACCAAATTCA ACCTATGACT ACTTCCTTT GCCTGCTGGG CTCTCAAAGA  
45         451     ATACTTCAAA TTCGAATGGA CATTATGAGA CAGCTGTTGA ACCTAAGTTT  
25         501     AATTCAAGTG GTACTCACTT TTCTAACTTA TCCAAAACAA CTTTCCACTG  
55         551     TTGCTTTCGG AGTGAGCAAG ATAGAAACTG CTCCTTATGT GCAGACAACA  
30         601     TTGAAGGAAA GACATTTGTT TCAACAGTAA ATTCTTTAGT TTTTCAACAA  
65         651     ATAGATGCAA ACTGGAACAT ACAGTGCTGG CTAAAAGGAG ACTTAAAATT  
70         701     ATTCACTCTGT TATGTGGAGT CATTATTTAA GAATCTATTG AGGAATTATA  
35         751     ACTATAAGGT CCATCTTTA TATGTTCTGC CTGAAGTGTGTT AGAAGATTCA  
80         801     CCTCTGGTTC CCCAAAAAAGG CAGTTTCAG ATGGTTCACT GCAATTGCAG  
40         851     TGTTCACGAA TGTTGTGAAT GTCTTGTGCC TGTGCCAACCA GCCAAACTCA  
90         901     ACGACACTCT CCTTATGTGT TTGAAAATCA CATCTGGTGG AGTAATTTTC  
95         951     CAGTCACCTC TAATGTCAGT TCAGCCCATA AATATGGTGA AGCCTGATCC  
45         1001    ACCATTAGGT TTGCATATGG AAATCACAGA TGATGGTAAT TTAAAGATTT  
105       1051    CTTGGTCCAG CCCACCATTG GTACCATTTC CACTTCATA TCAAGTGAAA  
50         1101    TATTCAGAGA ATTCTACAAC AGTTATCAGA GAAGCTGACA AGATTGTCTC  
115       1151    AGCTACATCC CTGCTAGTAG ACAGTATACT TCCTGGGTCT TCGTATGAGG

1201 TTCAGGTGAG GGGCAAGAGA CTGGATGGCC CAGGAATCTG GAGTGACTGG  
1251 AGTACTCCTC GTGTCTTAC CACACAAGAT GTCATATACT TTCCACCTAA  
5 1301 AATTCTGACA AGTGTGGGT CTAATGTTTC TTTTCACTGC ATCTATAAGA  
1351 AGGAAAACAA GATTGTTCCC TCAAAAGAGA TTGTTGGTG GATGAATTAA  
1401 GCTGAGAAAA TTCCTCAAAG CCAGTATGAT GTTGTGAGTG ATCATGTTAG  
10 1451 CAAAGTTACT TTTTCAATC TGAATGAAAC CAAACCTCGA GGAAAGTTA  
1501 CCTATGATGC AGTGTACTGC TGCAATGAAC ATGAATGCCA TCATCGCTAT  
15 1551 GCTGAATTAT ATGTGATTGA TGTCAATATC AATATCTCAT GTGAAACTGA  
1601 TGGGTACTTA ACTAAAATGA CTTGCAGATG GTCAACCAGT ACAATCCAGT  
1651 CACTTGCGGA AAGCACTTTG CAATTGAGGT ATCATAGGAG CAGCCTTTAC  
20 1701 TGTCTGATA TTCCATCTAT TCATCCCATA TCTGAGGCCA AAGATTGCTA  
1751 TTTGCAGAGT GATGGTTTT ATGAATGCAT TTTCCAGCCA ATCTTCCTAT  
25 1801 TATCTGGCTA CACAATGTGG ATTAGGATCA ATCACTCTCT AGGTTCACTT  
1851 GACTCTCCAC CAACATGTGT CCTTCCTGAT TCTGTGGTGA AGCCACTGCC  
1901 TCCATCCAGT GTGAAAGCAG AAATTACTAT AACACATTGGA TTATTGAAAA  
30 1951 TATCTTGGGA AAAGCCAGTC TTTCCAGAGA ATAACCTTCA ATTCCAGATT  
2001 CGCTATGGTT TAAGTGGAAA AGAAGTACAA TGGAAGATGT ATGAGGTTA  
35 2051 TGATGCAAAA TCAAAATCTG TCAGTCTCCC AGTTCCAGAC TTGTGTGCAG  
2101 TCTATGCTGT TCAGGTGCGC TGTAAGAGGC TAGATGGACT GGGATATTGG  
2151 AGTAATTGGA GCAATCCAGC CTACACAGTT GTCATGGATA TAAAAGTTCC  
40 2201 TATGAGAGGA CCTGAATTT GGAGAATAAT TAATGGAGAT ACTATGAAAA  
2251 AGGAGAAAAA TGTCACTTTA CTTTGGAAC CCCTGATGAA AAATGACTCA  
45 2301 TTGTGCAGTG TTCAGAGATA TGTGATAAAC CATCATACTT CCTGCAATGG  
2351 AACATGGTCA GAAGATGTGG GAAATCACAC GAAATTCACT TTCCGTGGA  
2401 CAGAGCAAGC ACATACTGTT ACGGTTCTGG CCATCAATTG AATTGGTGCT  
50 2451 TCTGTTGCAA ATTTAATT AACCTTTCA TGGCCTATGA GCAAAGTAAA  
2501 TATCGTGCAG TCACTCAGTG CTTATCCTTT AAACAGCAGT TGTGTGATTG

2551 TTTCTGGAT ACTATCACCC AGTGATTACA AGCTAATGTA TTTTATTATT  
2601 GAGTGGAAAA ATCTTAATGA AGATGGTGAA ATAAAATGGC TTAGAATCTC  
5 2651 TTCATCTGTT AAGAAGTATT ATATCCATGA TCATTTATC CCCATTGAGA  
2701 AGTACCAAGTT CAGTCTTAC CCAATATTAA TGGAAGGAGT GGGAAAACCA  
10 2751 AAGATAATTAA ATAGTTTCAC TCAAGATGAT ATTGAAAAAC ACCAGAGTGA  
2801 TGCAGGTGAC TACAAGGACG ACGATGACAA GTAGGGATCC AGACATGATA  
2851 AGATACATTG ATGAGTTGG ACAACCCACA ACTAGAATGC AGTAAAAAAA  
15 2901 ATGCTTTATT TGTGAAATTG GTGATGCTAT TGCTTTATTT GTAACCAT

Recombinant Human OB Receptor Protein, Natural Splice Variant  
amino acid sequence (Seq. ID. No. 13)

5    1    MICQKFCVVL LHWEFIYVIT AFNLSYPITP WRFKLSCMPP NSTYDYFLLP  
      51    AGLSKNTSNS NGHYETAVEP KFNSSGTHFS NLSKTTFHCC FRSEQDRNCS  
    10 101    LCADNIEGKT FVSTVNSL VF QQIDANWNIQ CWLKGDLKLF ICYVESLFKN  
      151    LFRNYNYKVH LLYVLPEVLE DSPLVPQKGS FQMVHCNC SV HECCECLVPV  
    20 201    PTAKLNDTLL MCLKITSGGV IFQSPLMSVQ PINMVKPDP P LGLHMEITDD  
      251    GNLKISWSSP PLVPFPLQYQ VKYSENSTTV IREADKIVSA TSLLVDSILP  
    30 301    GSSYEVQVRG KRLDGPGIWS DWSTPRVF TT QDVIYFPPKI LTSVGSNVSF  
      351    HCIYKKENKI VPSKEIVWWM NLAEKIPQSQ YDVVSDHVSK VTFFNLNETK  
    20 401    PRGKFTYDAV YCCNEHECHH RYAELYVIDV NINISCETDG YLTKMTCRWS  
      451    TSTIQSLAES TLQLRYHRSS LYCSDIPI SH PISEPKDCYL QSDGFYECIF  
    25 501    QPIFLLSGYT MWIRINHSLG SLDSPPTCVL PDSVVKPLPP SSVKAEITIN  
      551    IGLLKISWEK PVFPENN LQF QIRYGLSGKE VQWKMYEVYD AKSKSVSLPV  
    30 601    PDLCAVYAVQ VRCKRLDGLG YWSNWSNPAY TVVMDIKVPM RGPEFWRIIN  
      651    GDTMKKEKNV TLLWKPLMKN DSLCSVQRYV INHHTSCNGT WSEDVGNHTK  
    35 701    FTFLWTEQAH TVTVLAINSI GASVANFNLT FSWPMSKVNI VQSL SAYPLN  
      751    SSCVIVSWIL PSDYKLMYF IIEWKNLNED GEIKWLRSS SVKKYYIHGK  
    801    FTIL

Human OB Receptor Protein, Natural Splice Variant DNA (Seq. ID.  
No. 14)

1        GCGGCCGCCA GTGTGATGGA TATCTGCAGA ATTGGCTTT CTCTGCCTTC  
5        51        GGTGAGTTG GACCCCCGGA TCAAGGTGTA CTTCTCTGAA GTAAGATGAT  
10      101      TTGTCAAAAA TTCTGTGTGG TTTTGTACA TTGGGAATT ATTATGTGA  
15      151      TAACTGCGTT TAACTTGTCA TATCCAATT A C C C T T G G A G ATTTAAGTTG  
20      201      TCTTGCATGC CACCAAATT AACCTATGAC TACTCCTTT TGCCTGCTGG  
25      251      GCTCTCAAAG AATACTCAA ATTGAATGG ACATTATGAG ACAGCTGTTG  
30      301      AACCTAAGTT TAATTCAAGT GGTACTCACT TTTCTAACTT ATCCAAAACA  
35      351      ACTTTCCACT GTTGCTTTCG GAGTGAGCAA GATAGAAACT GCTCCTTATG  
40      401      TGCAGACAAC ATTGAAGGAA AGACATTGT TTCAACAGTA AATTCTTAG  
45      451      TTTTCAACA AATAGATGCA AACTGGAACA TACAGTGCTG GCTAAAAGGA  
50      501      GACTTAAAAT TATTCACTG TTATGTGGAG TCATTATTTA AGAATCTATT  
55      551      CAGGAATTAT AACTATAAGG TCCATCTTT ATATGTTCTG CCTGAAGTGT  
60      601      TAGAAGATTAC ACCTCTGGTT CCCCAAAAAG GCAGTTTCA GATGGTCAC  
65      651      TGCAATTGCA GTGTTCACGA ATGTTGTGAA TGTCTTGTGC CTGTGCCAAC  
70      701      AGCCAAACTC AACGACACTC TCCTTATGTG TTTGAAAATC ACATCTGGTG  
75      751      GAGTAATTT CCAGTCACCT CTAATGTCAG TTCAGCCCCTT AAATATGGTG  
80      801      AAGCCTGATC CACCATTAGG TTTGCATATG GAAATCACAG ATGATGGTAA  
85      851      TTTAAAGATT TCTTGGTCCA GCCCACCATT GGTACCATT CCACCTCAAT  
90      901      ATCAAAGTGAA ATATTCAAGAG AATTCTACAA CAGTTATCAG AGAAGCTGAC  
95      951      AAGATTGTCT CAGCTACATC CCTGCTAGTA GACAGTATAC TTCCTGGGTC  
100     1001     TTCGTATGAG GTTCAGGTGA GGGGCAAGAG ACTGGATGGC CCAGGAATCT  
105     1051     GGAGTGACTG GAGTACTCCT CGTGTCTTA CCACACAAAGA TGTCAATATAC  
110     1101     TTTCCACCTA AAATTCTGAC AAGTGTGGG TCTAATGTTT CTTTCACTG  
115     1151     CATCTATAAG AAGGAAAACA AGATTGTTCC CTCAAAAGAG ATTGTTGGT  
120     1201     GGATGAATTT AGCTGAGAAA ATTCCCTAAA GCCAGTATGA TGTTGTGAGT

1251 GATCATGTTA GCAAAGTTAC TTTTTCAAT CTGAATGAAA CCAAACCTCG  
1301 AGGAAAGTTT ACCTATGATG CAGTGTACTG CTGCAATGAA CATGAATGCC  
5 1351 ATCATCGCTA TGCTGAATTA TATGTGATTG ATGTCAATAT CAATATCTCA  
1401 TGTGAAACTG ATGGGTACTT AACTAAAATG ACTTGCAGAT GGTCAACCAG  
1451 TACAATCCAG TCACTTGCAG AAAGCACTTT GCAATTGAGG TATCATAGGA  
10 1501 GCAGCCTTTA CTGTTCTGAT ATTCCATCTA TTCATCCCCT ATCTGAGCCC  
1551 AAAGATTGCT ATTGCAAGAG TGATGGTTTT TATGAATGCA TTTTCCAGCC  
15 1601 AATCTTCCTA TTATCTGGCT ACACAATGTG GATTAGGATC AATCACTCTC  
1651 TAGGTTCACT TGACTCTCCA CCAACATGTG TCCTTCCTGA TTCTGTGGTG  
1701 AAGCCACTGC CTCCATCCAG TGTGAAAGCA GAAATTACTA TAAACATTGG  
20 1751 ATTATTGAAA ATATCTTGGG AAAAGCCAGT CTTTCCAGAG AATAACCTTC  
1801 AATTCCAGAT TCGCTATGGT TTAAGTGGAA AAGAAGTACA ATGGAAGATG  
25 1851 TATGAGGTTT ATGATGCAAA ATCAAAATCT GTCAGTCTCC CAGTTCCAGA  
1901 CTTGTGTGCA GTCTATGCTG TTCAGGTGCG CTGTAAGAGG CTAGATGGAC  
1951 TGGGATATTG GAGTAATTGG AGCAATCCAG CCTACACAGT TGTCATGGAT  
30 2001 ATAAAAGTTC CTATGAGAGG ACCTGAATT TGGAGAATAA TTAATGGAGA  
2051 TACTATGAAA AAGGAGAAAA ATGTCACTTT ACTTTGGAAG CCCCTGATGA  
35 2101 AAAATGACTC ATTGTGCAGT GTTCAGAGAT ATGTGATAAA CCATCATACT  
2151 TCCTGCAATG GAACATGGTC AGAAGATGTG GGAAATCACA CGAAATTAC  
2201 TTTCCTGTGG ACAGAGCAAG CACATACTGT TACGGTTCTG GCCATCAATT  
40 2251 CAATTGGTGC TTCTGTTGCA AATTTAATT TAACCTTTTC ATGGCCTATG  
2301 AGCAAAGTAA ATATCGTGCA GTCACTCAGT GCTTATCCTT TAAACAGCAG  
45 2351 TTGTGTGATT GTTCCTGGAA TACTATCACC CAGTGATTAC AAGCTAATGT  
2401 ATTTTATTAT TGAGTGGAAA AATCTTAATG AAGATGGTGA AATAAAATGG  
2451 CTTAGAATCT CTTCATCTGT TAAGAAGTAT TATATCCATG GTAAGTTAC  
50 2501 TATACTT

While the present invention has been described in terms of preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations which come within the scope of the invention as claimed.

## CLAIMS

10

1. An OB receptor protein preparation containing an OB receptor protein, optionally in a pharmaceutically acceptable formulation, said OB receptor protein having part or all of the amino acid sequence according to Seq. ID No. 1 and one or more of the biological properties of naturally occurring OB receptor protein.

2. An OB receptor protein preparation containing an OB receptor protein, optionally in a pharmaceutically acceptable formulation, wherein said OB receptor protein amino acid sequence is selected from among amino acid sequences (according to Seq. ID No. 1):

- (a) 1-896;
- (b) 22-896 optionally with an N-terminal methionyl residue;
- (c) 23-896 optionally with an N-terminal methionyl residue;
- (d) 29-896 optionally with an N-terminal methionyl residue;
- (e) 1-839;
- (f) 22-839 optionally with an N-terminal methionyl residue;
- (g) 29-839 optionally with an N-terminal methionyl residue;
- (h) 1-841;

- (i) 22-841 optionally with an N-terminal methionyl residue;
- (j) 23-841 optionally with an N-terminal methionyl residue;
- 5 (k) 29-841 optionally with an N-terminal methionyl residue;
- (l) 1-891;
- (m) 22-891 optionally with an N-terminal methionyl residue;
- 10 (n) 23-891 optionally with an N-terminal methionyl residue;
- (o) 29-891 optionally with an N-terminal methionyl residue;
- (p) of subparts (l) through (o) further having the C-terminal amino acids, beginning at position 892, of OB receptor B (Seq. ID No. 3) or C (Seq. ID. No. 5); and,
- 15 (q) a chemically modified derivative of any of subparts (a) through (p).

20

- 3. An OB receptor protein preparation of claim 2 wherein said OB receptor protein is further selected from among the OB receptor proteins of subparts (l) through (o) further having the C-terminal amino acids, beginning at position 892, of OB receptor protein D (Seq. ID No. 7).

- 4. An OB receptor protein preparation of claim 2 wherein said OB receptor protein is further selected from among the OB receptor proteins of subparts (l) through (o) further having substituted the C-terminal amino acids, beginning at position 799, G K F T I L (Seq. ID No. 13).

5. An OB receptor protein preparation according to any of claims 1 through 4, wherein the extracellular domain of said OB receptor protein is modified, said modification selected from among:
- (a) deletion of all or part of the random coil domain;
  - (b) modification of one or both "WSXWS" boxes by substitution of the first serine with another amino acid;
  - (c) modification of one or both "WSXWS" boxes by substitution of the last serine with another amino acid; and
  - (d) modification of one or both "WSXWS" boxes by substitution of the first tryptophan with another amino acid.

6. A DNA molecule encoding an OB receptor protein according to any of claims 1-5 selected from the group consisting of:

- (a) the DNA sequences set forth in Seq. ID nos. 2, 4, 6, 8, 11, 12, and 14;
- (b) a DNA which selectively hybridizes to a DNA of subpart (a); and
- (c) a DNA which, but for the degeneracy of the genetic code would hybridize to a DNA of subpart (a) or (b).

7. A biologically functional viral or plasmid vector containing a DNA of claim 6.

8. A procaryotic or eucaryotic host cell containing the vector of claim 7.

35 9. A host cell modified so that expression of endogenous OB receptor protein is enhanced.

10. A host cell of claim 9 which is an isolated human host cell.

5           11. A process for producing an OB receptor protein comprised of culturing, under suitable conditions, a host cell according to any of claims 8, 9 or 10, obtaining the OB receptor produced, and optionally preparing a pharmaceutical composition  
10 containing said OB receptor.

12. A method of treating an individual for a therapeutic disorder selected from among obesity, diabetes, high blood lipid levels, and high cholesterol  
15 levels comprised of administering a therapeutic amount of an OB receptor protein preparation containing an OB receptor protein according to any of claims 1-5, or produced by the process according to claim 11.

20           13. A method of treating an individual for weight loss or weight maintenance for solely cosmetic purposes comprised of administering an effective amount of an OB receptor preparation containing an OB receptor protein according to any of claims 1-5, or produced by  
25 the process according to claim 11.

14. Use of an OB receptor protein according to claims 1-5, or produced by the process of claim 11, for manufacturing a medicament for the treatment of  
30 obesity, diabetes, high blood lipid levels, or high cholesterol levels.

15. An OB protein/OB receptor protein complex preparation, containing an OB protein moiety and an OB receptor protein moiety, optionally in a pharmaceutically acceptable formulation, wherein:

(a) said OB receptor protein is selected from among those set forth in any of claims 1 and 2;

10 (b) said OB protein moiety is selected from among:

(i) a naturally occurring OB protein; and,

(ii) a non-naturally occurring OB protein, analog or derivative thereof.

15 16. An OB protein/OB receptor protein complex preparation of claim 15 wherein said OB receptor protein is selected from among those set forth in any of claims 3, 4, and 5.

20 17. A method of treating an individual for a therapeutic disorder selected from among obesity, diabetes, high blood lipid levels, and high cholesterol levels comprised of administering a therapeutic amount 25- of an OB protein/OB receptor protein complex preparation of claims 15 or 16.

30 18. A method of claim 17 wherein said OB protein/OB receptor protein complex preparation is formed in vivo by administering, into a patient, a first population of cells expressing an OB protein, and a second population of cells expressing an OB receptor protein.

19. A method of treating an individual for weight loss or weight maintenance for solely cosmetic purposes comprised of administering a therapeutic amount 5 of an OB protein/OB receptor protein complex preparation containing an OB receptor protein moiety according to any of claims 1-5, or produced by the process according to claim 11.
- 10 20. Use of an OB protein/OB receptor protein complex preparation, according to claims 15 or 16, for manufacturing a medicament for the treatment of obesity, diabetes, high blood lipid levels, or high cholesterol levels.
- 15